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13. ABSTRACT (Maximum 200 Words) Individual studies have been conducted to determine the identity of the urinary metabolites of artelinic acid eliminated by dogs and to investigate the dose-related pharmacodynamic effects of artelinic acid, as assessed by signs of clinical and pathological toxicity, in male and female rats and dogs. Using LC/MS/MS methodology, glucuronides of artelinic acid, hydroxy artelinic acid, DQHS, and hydroxy DQHS were tentatively identified in urine collected from dogs dosed with artelinic acid. In rats given 14 consecutive daily oral doses of artelinic acid of 0, 10, 20, 40, 80, or 320 mg/kg/day or IM doses of 12.5 mg/kg/day of arteether, body weight loss and mortality were observed at an artelinic acid dose of 320 mg/kg/day. Biologically relevant changes in hematology parameters observed on Day 21 included: increases in mean reticulocyte counts at artelinic acid doses of 20, 40, 80, and 320 mg/kg/day; and mild increases in erythrocyte MCV values at artelinic acid doses of 20 and 80 mg/kg/day. A biologically relevant decrease in mean ALP activity was also observed in rats in the 320 mg/kg/day dose group. At artelinic acid doses of 40 and 80 mg/kg/day and an arteether dose of 12.5 mg/kg/day, neuropathological lesions observed in the hind brain consisted of neuronal degeneration of the trapezoid nucleus; at an artelinic acid dose of 320 mg/kg/day, the lesions also included multiple nuclei in the hind brain. In dogs given 14 consecutive daily oral doses of artelinic acid of 0, 20, 40, 80, or 320 mg/kg/day or IM doses of 20 mg/kg/day of arteether, body weight loss was noted for dogs in the 320 mg/kg/day artelinic acid and the arteether dose groups. Potentially drug-related decreases in RBC, hemoglobin, hematocrit, and/or reticulocyte counts were observed in the 20, 40, 80, and 320 mg/kg/day artelinic acid and the arteether dose groups. Decreased albumin and albumin/globulin ratios and/or increased globulin concentrations were also observed in the 80 and 320 mg/kg/day artelinic acid and the arteether dose groups. No drug-related histopathological lesions were observed in either the brain or other tissues collected from dogs in any of the artelinic acid dose groups. It is planned that the results of these preliminary pharmacodynamic studies with orally administered artelinic acid will be extended during subsequent definitive investigations.			
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Table of Contents

	<u>Page</u>
Cover Page	1
Standard Form 298	2
1.0 Introduction	4
2.0 Research Accomplishments For Each Task Order	5
2.1 Task Order SR98-2: Bioavailability, Pharmacokinetics, and Identification of Metabolites of [¹⁴ C]Artelinic Acid in Dogs.....	5
2.2 Task Order SR98-3: Effect of Artelinic Acid on Rats after Oral Administration for 14 Days.....	7
2.3 Task Order SR99-1: Effect of Artelinic Acid on Dogs after Oral Administration for 14 Days.....	10
3.0 Key Research Accomplishments	16
4.0 Reportable Outcomes	16
5.0 Conclusions	17
6.0 References	18
Tables.....	19
Figures.....	40

1.0 INTRODUCTION

The work scope of this contract involves the performance of studies in rats and dogs on the pharmacokinetic and pharmacodynamic properties of drugs under clinical development by the U.S. Army Medical Research and Development Command. The pharmacokinetic aspect of these studies involves an investigation of the absorption, disposition, metabolism (biotransformation) and elimination of test compounds in experimental animals. The pharmacodynamic aspect involves relating certain measured parameters, for example, the production of methemoglobin, to blood and plasma levels of the test compound and/or its metabolites, or assessing toxicological parameters such as clinical signs and mortality occurring after administration of a test compound. The information derived from these studies is intended to provide a data base for establishing an appropriate species and appropriate doses for subsequent subchronic and chronic toxicity studies, to predict for possible organ toxicities which might occur, and to generate data required by the Food and Drug Administration prior to submission of a Notice of Claimed Investigational Exemption for a New Drug (IND) and New Drug Applications, Human Use (NDA).

During the past year of the contract, pharmacokinetic/pharmacodynamic studies were conducted with the anti-malarial agent, artelinic acid. The scope of the work accomplished during the past year included:

- Task Order SR98-2: Bioavailability, Pharmacokinetics, and Identification of Metabolites of [¹⁴C]Artelinic Acid in Dogs.* During work accomplished under this task order, tentative identification of the major urinary metabolites of [¹⁴C]artelinic acid excreted by dogs was accomplished.
- Task Order SR98-3: Effect of Artelinic Acid on Rats after Oral Administration for 14 Days.* During a preliminary investigation, the dose-related pharmacodynamic effects of artelinic acid in rats, as assessed by signs of clinical and pathological toxicity, following daily administration of artelinic acid by oral gavage for 14 consecutive days were investigated. The overall goal of the study is to determine a no-effect dose level, target organ toxicity, and the reversibility of target organ toxicity.
- Task Order SR99-1: Effect of Artelinic Acid on Dogs after Oral Administration for 14 Days.* During a preliminary investigation, the dose-related pharmacodynamic effects of artelinic acid in dogs, as assessed by signs of clinical and pathological toxicity, following daily administration of artelinic acid by oral gavage for 14 consecutive days were investigated. This included determination of a no-effect dose level, target organ toxicity, and the reversibility of target organ toxicity.

2.0 RESEARCH ACCOMPLISHMENTS FOR EACH TASK ORDER

2.1 Task Order SR98-2: Bioavailability, Pharmacokinetics, and Identification of Metabolites of [¹⁴C]Artelinic Acid in Dogs

2.1.1 Objectives. The objectives of this task order were to investigate, in both male and female beagle dogs, the time concentration profiles of artelinic acid and its metabolites in whole blood, plasma, urine, and feces following IV and PO administration of [¹⁴C]artelinic acid; to determine the oral bioavailability of artelinic acid; and to elucidate the identity of the major metabolites of artelinic acid.

During this study, 4 male and 4 female beagle dogs were administered a single IV or PO dose of 10 mg/kg of [¹⁴C]artelinic acid. The dose formulation for IV administration was a solution prepared in 0.5% sodium carbonate/saline. The dose formulation for PO administration was prepared as a suspension in 1% carboxymethylcellulose:0.5% Tween 80. At various times through 192 hours after administration of the IV or PO dose, blood/plasma, urine, and feces were collected from each animal and then assayed for total radioactivity using liquid scintillation counting, unchanged parent compound using LC/MS, and metabolites using HPLC with in-line radiodetection and LC/MS/MS. Pharmacokinetic parameters were calculated from blood and plasma concentrations of radioactivity and unchanged artelinic acid using WinNonlin (Scientific Consulting, Inc., Apex, NC).

The results of the analyses of the samples (both radioanalyses and chromatographic analyses) and the pharmacokinetic evaluations of the data were reported in the 1999 Annual Contract Report. During the past year of the contract, a draft report detailing the results of the in-life aspects of the study was submitted to the Contracting Officer's Representative for review. In addition, during the past year of the contract, the identity of the urinary metabolites of artelinic acid was investigated. These investigations are described below.

2.1.2 Methods. Based in part on work accomplished by Maggs and coworkers (1), it was predicted that the metabolites of artelinic acid might include various glucuronides of the free acid and hydroxylated derivatives. Following this approach, a Perkin Elmer Sciex API 3000 LC/MS/MS instrument was used to screen urine samples for expected metabolites of artelinic acid by neutral loss, by scanning in single quadrupole mode, and by mixed reaction monitoring.

2.1.3 Results. Utilizing the above methods, several urinary metabolites of artelinic acid were tentatively identified. The identifications were based on adding mass units, fragmentation patterns, and literature on the known metabolites of artelinic acid and related compounds. The metabolites tentatively identified included the following:

Glucuronide of DQHS: A urine sample collected from a dog dosed with artelinic acid was injected into an Aquasil C18 guard column on the LC/MS/MS instrument and the effluent was monitored for the product ions of $m/z=459$ in the negative mode. $M/z=459$ would correspond to $m/z=283$ (the DQHS negative ion) + glucuronide ($m/z=176$). The actual ion products observed were $m/z=283$ and $m/z=193$ (Figure 1). The $m/z=193$ corresponded to the open form of the

glucose molecule and suggested the molecule had been conjugated to a hydroxyl. In the positive ion mode, the expected ion was $m/z=499$, which would correspond to the K salt of the glucuronide. The product ion observed was $m/z=323$, which corresponded to DQHS + K (284+39). Based on these and other data, the major urinary metabolite of artelinic acid was identified as a glucuronide of DQHS.

Glucuronides of hydroxy DQHS: A urine sample collected from a dog dosed with artelinic acid was injected into an Aquasil C18 guard column on the LC/MS/MS instrument and the effluent was monitored for the product ions of $m/z=475$ in the negative mode. $M/z=475$ would correspond to $m/z=283$ (DQHS) + 16 (oxygen) + 176 (glucuronide). The product ions observed were $m/z=193$ (open glucose), $m/z=299$ (hydroxy DQHS), and a trace of $m/z=175$ (Figure 2). The position of the hydroxyl group could not be assigned based on this fragmentation. As shown in Figure 3, an additional metabolite, having a slightly different retention time (3.34 minutes, instead of 2.90 minutes), was found to yield the same fragmentation, producing $m/z=193$, $m/z=113$, and $m/z=175$ fragments. This fragmentation pattern suggested that one metabolite was the result of conjugation on the hydroxy group added to the ring and the other metabolite was the result of conjugation of the hydroxy group of DQHS.

Glucuronide of artelinic acid: A urine sample collected from a dog dosed with artelinic acid was injected into an Aquasil C18 guard column on the LC/MS/MS instrument and the effluent was monitored for the product ions of $m/z=593$ in the negative mode. $M/z=593$ corresponded to the negative ion of artelinic acid ($m/z=417$) + glucuronide ($m/z=175$). It was proposed that glucuronide conjugation would occur on the carboxylic acid group of artelinic acid, thus, no $m/z=193$ was expected for the open glucose ion. The actual ion products observed were $m/z=417$ (artelinic acid negative ion), $m/z=175$ (glucuronide), and 113 (a fragment associated with glucuronides) (Figure 4).

Glucuronide of hydroxy artelinic acid: A urine sample collected from a dog dosed with artelinic acid was injected into an Aquasil C18 guard column on the LC/MS/MS and the effluent was monitored for the product ions of $m/z=609$ in the negative mode. $M/z=609$ would correspond to the negative ion of artelinic acid ($m/z=417$) + oxygen (16) + glucuronide (176). The actual products observed were $m/z=433$ and $m/z=175$ (Figure 5). These corresponded to $417 + 16 = 433$ (hydroxylated artelinic acid) and 175 (glucuronide closed ring). The absence of $m/z=193$ suggested the conjugation occurred on the carboxylate group of artelinic acid.

Possible sulfate conjugate: A metabolite was observed in urine using a product ion scan of $m/z=514$ in the negative mode. The product ions observed were $m/z=212$, $m/z=301$, and $m/z=434$. These data suggested that the metabolite was a sulfate conjugate of artelinic acid. $M/z=514$ would correspond to 418 (artelinic acid with no charge) + 80 (sulfate with negative charge) + 16 (oxygen). The $m/z=434$ would correspond to the hydroxylated artelinic acid fragment.

2.1.4 Discussion. The results of these metabolite analyses provide additional information regarding the metabolism of artelinic acid. Previous investigators have shown that artelinic acid is metabolized to dihydroqinghaosu (DQHS) and also undergoes hydroxylation to dihydroartemisinin (2,3). The data obtained during the current study also indicate artelinic acid

derivatives undergo conjugation reactions. Thus, both Phase I and Phase II reactions appear to be involved in the metabolism of artelinic acid in dogs.

2.2 Task Order SR98-3: Effect of Artelinic Acid on Rats after Oral Administration for 14 Days.

2.2.1 Objective. The objective of this study is to determine the dose-related pharmacodynamic effects of artelinic acid, as assessed by signs of clinical and pathological toxicity, in rats following daily administration for 14 consecutive days. This includes determination of a no-effect dose level, target organ toxicity, and the reversibility of target organ toxicity. The study includes two phases: an initial dose range finding evaluation to select for an appropriate dose(s) of artelinic acid that produces a measurable pharmacodynamic effect; and a subsequent definitive study to fully characterize any dose-related pharmacodynamic effects of artelinic acid. In each phase of the study, a positive control group, that receives arteether, is included. Assessments include: examinations of individual animals for clinical signs of toxicity, body weight measurements, and histopathological evaluations of tissues. During the past year of the contract, the pilot evaluation phase of this study was completed.

2.2.2 Methods. Male and female Sprague-Dawley rats (Charles River Laboratories, Raleigh, NC) were used. Dose formulations of artelinic acid were prepared as suspensions in 1% carboxymethylcellulose:0.2% Tween 80 in sterile water to contain 2, 4, 8, 16, or 64 mg/mL of artelinic acid. Dose formulations of arteether were prepared as solutions in sesame oil containing 25 mg/mL of arteether. The vehicle formulation consisted of 1% carboxymethyl cellulose:0.2% Tween 80.

Oral gavage (PO) doses of 0 (vehicle only), 10, 20, 40, 80, or 320 mg/kg/day of artelinic acid or an IM dose of 12.5 mg/kg/day of arteether (the positive control) were administered to groups of two or four male and female rats once each day for 14 consecutive days as follows:

Group	Compound	Route	mg/kg/day	No. ♂	No. ♀
V	None	PO	0	2	2
A	Artelinic acid	PO	10	4	4
B	Artelinic acid	PO	20	4	4
C	Artelinic acid	PO	40	4	4
D	Artelinic acid	PO	80	4	4
E	Artelinic acid	PO	320	4	4
F	Arteether	IM	12.5	2	2

On Days 1 through 21, animals were observed at least twice daily for mortality and clinical signs of toxicity; in particular, appearance, gait, activity, level of arousal, and motor coordination were assessed.

Clinical pathology testing was conducted on all animals at terminal sacrifice on Day 21. Both hematology and clinical chemistry evaluations were performed. Prior to sample collection, animals were fasted overnight with access to water. Rats were anesthetized with CO₂/O₂, and blood was collected from the retro-orbital sinus into tubes containing EDTA (hematology evaluations) and into tubes containing no anticoagulant (clinical chemistry evaluations). The samples were used for the following determinations:

Hematology:

Hemoglobin (HGB)
Hematocrit (HCT)
Total leukocyte count (WBC)
Erythrocyte count (RBC)
Platelet count (PLT)
Reticulocyte counts (RETIC)
Differential leukocyte count
Mean corpuscular volume (MCV)
Mean corpuscular hemoglobin (MCH)
Mean corpuscular hemoglobin concentration (MCHC)

Clinical Chemistry:

Glucose (GLUC)
Creatinine (CREA)
Serum aspartate aminotransferase (AST)
Sodium (Na)
Potassium (K)
Chloride (Cl)
Blood urea nitrogen (BUN)
Serum alanine aminotransferase (ALT)
Alkaline phosphatase (ALP)
Total protein (TP)
Blood urea nitrogen/creatinine ratio (BUN/CREA ratio)

At sacrifice on Day 21, individual animals were anesthetized with pentobarbital (40-50 mg/kg, IP) and then perfused with a fixative of 1% glutaraldehyde and 4% paraformaldehyde buffered in a phosphate buffer. After fixation was complete, the central nervous system of each animal was removed for neurotoxicological evaluation.

For the neurotoxicological evaluation, tissues obtained from rats in the high-dose artelinic acid group (320 mg/kg/day), the vehicle control group, and the positive control group (arteether) were processed first. Tissues were embedded in paraffin and sectioned (5 µm sections). The brain was trimmed to include areas of the cerebral cortex, basal ganglia, thalamus, midbrain, cerebellum, and brainstem including major nuclei and pons. The slides were stained with haematoxylin and eosin (H & E), Luxol Fast Blue and a Nissl stain. Individual slides were

evaluated by a pathologist and lesions were graded on a five point scale with 0 as normal and 4 as the most severe.

In that this was a preliminary evaluation and only a limited number of animals were included in each dose group, no statistical analyses of the data were performed.

2.2.3 Results

Mortality. For animals in the 320 mg/kg/day dose group, one female rat was found dead on the morning of Day 10 of the dosing period and the other female rat in this dose group was found dead on Day 12.

No mortality was observed for either male or female rats given daily doses of 10, 20, 40, or 80 mg/kg/day of artelinic acid PO or 12.5 mg/kg/day of arteether IM.

Clinical Signs. No clinical signs of toxicity were observed for either male or female rats in the 10, 20, 40 or 80 mg/kg/day artelinic acid dose groups or in the 12.5 mg/kg/day arteether dose group.

For the female rats in the 320 mg/kg/day artelinic acid dose group, prior to death, both animals appeared emaciated and hunched (starting on Day 8 or 9) and one of these rats also exhibited a nose and eye discharge (starting on Day 9). Emaciation and hunching, starting on Day 7, were also observed for the male rats given 320 mg/kg/day.

Body Weights. A summary of the body weight data is presented in Table 1. Slight body weight losses (<10 g) or the absence of body weight gain were noted between Day 1 and Day 14 for female rats in the 80 mg/kg/day dose group. Body weight reductions (20-30% body weight loss) were noted between Day 1 and Day 14 (or the day of death) for the two male and two female rats given 320 mg/kg/day of artelinic acid. Animals in the other dose groups did not display body weight loss.

Hematology and Clinical Chemistry. A summary of the hematology data is presented in Table 2. Potentially drug-related changes in hematology parameters that were observed on Day 21 and considered to be biologically relevant included: increases in mean reticulocyte counts in male and/or female rats in the 20, 40, 80, and 320 mg/kg/day artelinic acid dose groups; and mild increases in erythrocyte MCV values in male rats in the 20 and 80 mg/kg/day artelinic acid dose groups and in female rats in the 80 mg/kg/day dose group.

A summary of the clinical pathology data is presented in Table 3. The only potentially drug-related change in clinical chemistry parameters was a biologically relevant decrease in mean ALP activity (49% of vehicle control group value) in male rats in the 320 mg/kg/day artelinic acid dose group.

Histopathology. Histopathological findings for brain are listed in Table 4. "Normal" indicates that all sections of the hind brain were within normal limits. All observations of "neuron degeneration" were for the trapezoid nucleus except where noted. The neuron

degeneration was characterized by loss of Nissl staining, swelling, and margination of the nucleus. These cells appeared to have red/brown cytoplasm on H & E section. More severe lesions had clumping of eosinophilic debris in some cells, minimal evidence of satellitosis, and minimal gliosis. The severity of ratings of +1 to +4 were an indication of the extent of the lesion with more neurons involved at the higher severity grade. At the +1 severity there were no more than 1 to 3 or 4 neurons involved in a section of the trapezoid nucleus.

As shown in Table 4, lesions were present in rats given the positive control agent, arteether, and in rats given artelinic acid at doses of 40 mg/kg/day and above. At artelinic acid doses of 40 and 80 mg/kg/day, the lesions consisted of neuronal degeneration of the trapezoid nucleus; at an artelinic acid dose of 320 mg/kg/day, the lesions included neuronal degeneration of the trapezoid nucleus and multiple nuclei in the hind brain.

2.2.4. Discussion. The results of this preliminary evaluation in rats given oral suspensions of artelinic acid for 14 consecutive days indicated that no mortality, no overt clinical signs of toxicity, and minimal effects on body weight gain were observed in animals given doses of artelinic acid of 80 mg/kg/day or lower; histopathological lesions were observed at artelinic acid dose levels of 40 mg/kg/day and higher; hematological changes were observed at artelinic acid dose levels of 20 mg/kg/day and higher. The histopathological lesions observed in rats given intramuscular arteether were consistent with the previously reported neurotoxicity of this compound. Thus, from these data, information on a "no effect" dose for the production of neurotoxicity in rats by artelinic acid was obtained and a "no effect" dose for observable hematological changes was also obtained. The results of this evaluation will be used to select doses for the definitive phase of the study.

2.3 Task Order SR99-1: Effect of Artelinic Acid on Dogs after Oral Administration for 14 Days.

2.3.1 Objectives. The objective of this study is to determine the dose-related pharmacodynamic effects of artelinic acid, as assessed by signs of clinical and pathological toxicity, in dogs following daily administration for 14 consecutive days. This includes determination of a no-effect dose level, target organ toxicity, and the reversibility of target organ toxicity. The study includes two phases: an initial dose range finding evaluation to select for an appropriate dose(s) of artelinic acid that produces a measurable pharmacodynamic effect; and a subsequent definitive study to fully characterize any dose-related pharmacodynamic effects of artelinic acid. In each phase of the study, a positive control group, that receives arteether, is included. Assessments include: examinations of individual animals for clinical signs of toxicity, body weight measurements, and histopathological evaluations of tissues. During the past year of the contract, the pilot evaluation phase of this study was completed.

2.3.2 Methods. Male and female dogs (Marshall Farms, Inc., South Rose, NY) were used in the study. Dose formulations of artelinic acid were prepared as suspensions in 1% carboxymethylcellulose:0.2% Tween 80 in sterile distilled water and were prepared to contain 2, 4, 8, or 32 mg/mL of artelinic acid. The corresponding vehicle formulation contained 1% (w/v)

carboxymethylcellulose:0.2% (v/v) Tween 80 in sterile distilled water. Dose formulations of arteether were prepared as solutions in sesame oil containing 100 mg/mL of arteether.

Groups of two male and two female dogs were given PO doses (gavage) of 0 (vehicle), 20, 40, 80, or 320 mg/kg/day of artelinic acid or an IM dose of 20 mg/kg/day of arteether (the positive control) once each day for 14 consecutive days as follows:

Group	Compound	Route	mg/kg/day	No. ♂	No. ♀
V	None	PO	0	2	2
A	Artelinic acid	PO	20	2	2
B	Artelinic acid	PO	40	2	2
C	Artelinic acid	PO	80	2	2
D	Artelinic acid	PO	320	2	2
E	Arteether	IM	20	2	2

On Days 1 through 21, dogs were observed at least twice daily for mortality/morbidity. On Days 1-14, a detailed clinical examination of each animal was performed within approximately 1-3 hours after dosing. In addition, on Days 1-14, animals were carefully assessed two times a day (with a minimum of 4 hours between each evaluation) for signs of central nervous system toxicity; changes in posture, activity, level of arousal, gait, breathing, and motor coordination were assessed. Body weights were obtained daily on Days 1-14 and prior to necropsy.

On Days -1, 7, and 15, a blood sample (3-4 mL) was collected from each dog/sex in dose groups A, B, C, D, and E; on Day 21, a blood sample (20 mL) was collected from each animal in these dose groups. The blood collection on Day 7 was made immediately prior to administration of the daily dose of artelinic acid or arteether; the time of blood collection on Day 21 was at the time of sacrifice. In addition, on Days 1 and 14, a blood sample (3-4 mL) was collected from each dog/sex in dose Groups A, B, C, D, and E prior to dosing and at the following times after dosing: 0.25, 0.5, 1, 2, 4, and 8 hours. Each blood sample was collected from the jugular vein into a tube containing heparin. Upon collection, each blood sample was centrifuged to obtain plasma. Plasma samples were stored at or below -70°C prior to shipment to Captain David Skanchy at WRAIR for analysis for artelinic acid or arteether concentrations.

Hematological and clinical chemistry evaluations were performed on animals on Days -1, 7, and prior to necropsy on Day 15 or 21. Prior to sample collection, animals were fasted overnight with access to water. Blood was drawn from a peripheral vein into tubes containing EDTA (hematology evaluations) and into tubes containing no anticoagulant (clinical chemistry evaluations). Blood samples were used for the following determinations:

Hematology:

Hemoglobin (HGB)
Hematocrit (HCT)
Total leukocyte count (WBC)
Erythrocyte count (RBC)
Platelet count (PLT)
Reticulocyte counts (RETIC)
Differential leukocyte count
Mean corpuscular volume (MCV)
Mean corpuscular hemoglobin (MCH)
Mean corpuscular hemoglobin concentration (MCHC)

Clinical Chemistry:

Glucose (GLUC)
Creatinine (CREA)
Serum aspartate aminotransferase (AST)
Sodium (Na)
Potassium (K)
Chloride (Cl)
Blood urea nitrogen (BUN)
Serum alanine aminotransferase (ALT)
Alkaline phosphatase (ALP)
Gamma glutamyl transferase (GGT)
Lactate dehydrogenase (LDH)
Creatine kinase (CK)
Total protein (TP)
Blood urea nitrogen/creatinine ratio (BUN/CREA ratio)

On Day 15 and on Day 21, one dog of each sex in each dose group was sacrificed by an overdose of a barbiturate followed by cardiac perfusion of a flush solution of 0.9% sodium chloride containing 1 mL of heparin (100 units/mL) and 1 mL of 1% sodium nitrate per liter. Immediately following the flush solution, each animal was perfused with an aldehyde fixative of 4% paraformaldehyde and 1% glutaraldehyde buffered with a phosphate buffer. The tissues listed below were removed for standard histopathological evaluation and the central nervous system was removed for neurotoxicological evaluation.

Adrenals
Aorta (aortic arch)
Bone, distal femoral head
Bone marrow (section from sternum and costochondral junction with rib)
Cecum
Colon
Duodenum
Esophagus
Eyes (including optic nerve and optic disk)
Gall bladder
Gross lesions
Heart (both atria, both ventricles, and intraventricular septum)

Ileum
Jejunum
Kidneys (2)
Liver (right medial lobe with section of gall bladder and left lateral lobe)
Lungs (left apical and left diaphragmatic lobes)
Lymph nodes (bronchial, mandibular, and mesenteric)
Mammary gland (left inguinal, when present)
Ovaries (2)
Pancreas
Pituitary
Prostate
Salivary gland
Sciatic nerve
Skeletal muscle
Skin [Non-frictional site (dorsal thorax); frictional site (elbow)]
Spinal cord (sections from the cervical and lumbar regions)
Spleen
Stomach (cardiac, pyloric, and fundic areas)
Testes (epididymides attached)
Thyroid and parathyroid (2)
Thymus
Tongue (including dorsal and lateral areas)
Tonsil (palatine)
Trachea
Ureter
Urinary bladder (fundus)

For the neurotoxicological evaluations on Days 15 and 21, the brains and spinal cord sections were removed at necropsy and stored in fresh perfusion fixative. Eyes were saved in Davidson's solution. All other tissues collected at necropsy were saved in 10% neutral buffered formalin.

Tissues were embedded in paraffin and sectioned (5 μ m sections). Representative sections were mounted on glass microscope slides and stained with H & E. Individual slides were evaluated by a pathologist and lesions were graded on a five point scale with 0 as normal and 4 as the most severe.

For the standard histopathological evaluation, only tissues obtained from dogs in the high artelinic acid dose group (320 mg/kg/day), the vehicle control group (0 mg/kg/day), and the positive control group (arteether) were processed.

In that this was a preliminary evaluation and only a limited number of animals were included in each dose group, no statistical analyses of the data were performed.

2.3.3 Results

Mortality. On Day 4, one female dog in the 320 mg/kg/day artelinic acid dose group displayed convulsions, emesis, prostration, and salivation shortly after administration of the daily

dose of artelinic acid. It appeared likely that the animal had aspirated at least some of the dosing formulation; this led to the ultimate sacrifice of the animal within a few hours after dosing.

One female dog in the 20 mg/kg/day arteether dose group displayed hypoactivity on Days 11-15, and then became extremely hyperexcited. The dog was euthanized at that point because of the threat of serious bodily injury to herself and/or the technical staff.

All other dogs in the study survived until their scheduled necropsy.

Clinical Signs. Sporadic diarrhea was noted for one or more dogs in each of the artelinic acid dose groups (20, 40, 80, or 320 mg/kg/day) and the arteether dose group (20 mg/kg/day). No other adverse clinical signs were observed in dogs given either 20, 40, or 80 mg/kg/day of artelinic acid.

For dogs given 320 mg/kg/day of artelinic acid, hypoactivity, starting on Day 11 of the 14-day dosing interval, was noted for one female dog in this dose group.

For dogs given 20 mg/kg/day of arteether, 1/2 female dogs in this dose group displayed ataxia on Days 10-12, and hypoactivity and prostration beginning on Day 10 and continuing through sacrifice on Day 15. Hypoactivity and subsequent hyperactivity were observed for the other female dog in this dose group and the latter is described above.

Body Weights. Individual body weights are presented in Table 5. Body weight loss (greater than 10%) was noted between Day 1 and the day of sacrifice for 1/2 male dogs and one female dog in the 320 mg/kg/day artelinic acid dose group, and for 1/2 male and 2/2 female dogs in the 20 mg/kg/day arteether dose group.

Hematology and Clinical Chemistry. Potentially drug-related decreases in RBC (70 to 80% of baseline values), hemoglobin, hematocrit, and/or reticulocyte counts ($\leq 33\%$ of baseline values) were observed in one or both male dogs in each of the 20, 40, 80, and 320 mg/kg/day artelinic acid dose groups, in one or two female dogs in each of the 40, 80, and 320 mg/kg/day artelinic acid dose groups and in dogs in the 20 mg/kg/day arteether dose group. Increased reticulocyte counts (> 2.0 fold baseline values) were observed on Day 21 in male dogs in the 40, 80, and 320 mg/kg/day artelinic acid dose groups and in the 20 mg/kg/day arteether dose groups; these increases were considered to be potentially drug related. In addition, drug-related decreases in leukocyte and neutrophil counts were observed in 1/2 male dogs and in 2/2 female dogs given 20 mg/kg/day of arteether; decreased lymphocyte counts were also observed in 1/2 female dogs in this dose group.

The predominant drug-related changes in clinical chemistry parameters were decreased albumin and albumin/globulin ratios and/or increased globulin concentrations in female dogs in the 80 and 320 mg/kg/day artelinic acid dose groups, and in male and female dogs in the 20 mg/kg/day arteether dose group.

Plasma Drug Levels. The results of the analyses of the plasma samples for levels of either artelinic acid or arteether have not yet been received from the laboratory responsible for these analyses.

Histopathology. For the neuropathological evaluations of brains, drug-related lesions were not observed in brain sections obtained from any dogs in either the 0, 20, 40, 80, or 320 mg/kg/day artelinic acid dose groups which were sacrificed on either Day 15 or 21. For dogs in the 20 mg/kg/day arteether dose group, drug-related lesions were observed; these included degenerating neurons in many areas of the hind brain.

Histopathologic evaluations were also conducted on the non-brain tissues obtained from male and female dogs in the vehicle control group (1% carboxymethylcellulose:0.2% Tween 80), the positive control group (arteether), and the high dose group (320 mg/kg/day artelinic acid). No lesions considered to be directly related to administration of artelinic acid (or arteether) were observed.

In the three artelinic acid high dose group dogs from the Day 15 or Day 21 scheduled sacrifices, thymic atrophy of moderate severity was seen in the male and female dogs sacrificed on Day 21. Thymus was not available for evaluation in the male dog sacrificed on Day 15. Mild testicular hypocellularity and cellular atypia were seen unilaterally in the male dog sacrificed on Day 15. Thymic and testicular changes similar to those seen in these dogs are occasionally observed in untreated beagles, and they were considered to be incidental in this study. Other lesions observed in the three artelinic acid high dose group dogs from the scheduled sacrifices included pituitary gland cyst (1/3) and mild skeletal muscle hypercellularity (1/3). These changes were also considered to be incidental.

One artelinic acid high dose group animal, which underwent moribund sacrifice on Day 4, had lung hemorrhage, lung edema, adrenal gland congestion, and bronchial lymph node hemorrhage of mild to marked severity, as well as diffuse hepatocellular necrosis of minimal severity. These were considered to be agonal lesions typical of those associated with acute death, the cause of which may have been related to aspiration of the dose formulation. This animal also had a pituitary gland cyst, which was considered an incidental finding.

In the absence of mortality in the remaining artelinic acid-treated dogs and of test article-related lesions in the three high dose group dogs from the scheduled sacrifices, evaluation of tissues dogs given lower doses of artelinic acid was not deemed necessary.

Lesions considered to be directly or indirectly test article related in the four dogs (2 male and 2 female) given arteether included bone marrow hyperplasia (2/4), spleen hematopoietic cell proliferation (3/4), spleen lymphoid depletion (2/4), lymph node lymphoid depletion (2/4), tonsil lymphoid depletion (1/4), liver hematopoietic cell proliferation (3/4), skeletal muscle inflammation (1/4), and oral mucosa erosion or ulcer (2/2). The remaining lesions seen in the arteether dogs were considered to be incidental or of uncertain cause.

2.3.4 Discussion. The results of this preliminary evaluation in dogs given artelinic acid as an oral suspension for 14 consecutive days indicated that no drug-related mortality was

observed at doses of 320 mg/kg/day and lower; with the exception of sporadic diarrhea, which was observed at all artelinic acid dose levels administered (20-320 mg/kg/day), no clinical signs of toxicity or body weight losses were observed for dogs given 20, 40, or 80 mg/kg/day of artelinic acid; no drug-related histopathological lesions were observed in either the brain or other tissues collected from dogs given from 20-320 mg/kg/day of artelinic acid; hematological changes were observed at all artelinic acid dose levels administered. The neuropathological lesions observed in dogs given arteether were consistent with the previously reported neurotoxicity of this compound. From these data, a highest "no effect" dose of artelinic acid for the production of neurohistopathological and other histopathological toxicity in dogs was not established; in addition, a 'no effect' dose for the production of hematological changes was not established. The results did indicate, however, that the dose of artelinic acid that effected hematological changes was at least an order of magnitude lower than the dose that effected histopathological changes.

3.0 KEY RESEARCH ACCOMPLISHMENTS

Key research accomplishments during the past year of the contract included:

- Mass spectral determination of the identity of urinary metabolites of artelinic acid in dogs.
- Characterization of the toxicological effects of 14-day administration of oral suspensions of artelinic acid to rats.
- Characterization of the toxicological effects of 14-day intramuscular administration of arteether to rats.
- Determination of a "no effect" dose for the production of the neurotoxicity by artelinic acid in rats.
- Characterization of the toxicological effects of 14-day administration of oral suspensions of artelinic acid to dogs.
- Characterization of the toxicological effects of 14-day intramuscular administration of arteether to dogs.
- Determination of a minimum "no effect" dose for the production of neurotoxicity by artelinic acid in dogs.

4.0 REPORTABLE OUTCOMES

Bossone, C.A., Q. Li, S. Mog, P. Lee, C. Ohrt, H. Chung, P.E. Noker, J.G. Page, and R. Brueckner. Toxicity of 14-day oral administration of artelinic acid. Abstract #311. 48th Annual Meeting of the American Society of Tropical Medicine and Hygiene, 1999.

5.0 CONCLUSIONS

The results of the LC/MS/MS analyses of urine collected from dogs administered artelinic acid provided additional information on the metabolism of artelinic acid in dogs. Previous investigators have shown that artelinic acid is metabolized to DQHS and dihydroartemisinin (2,3). The results obtained during the current investigations indicated that, in addition, to undergoing hydroxylation reactions, artelinic acid and its metabolite DQHS appear to be involved in conjugation reactions. Arteether has been reported to undergo similar metabolic transformations (1).

The results of the preliminary evaluation of the pharmacodynamic effects of artelinic acid in rats indicated that drug-related neuropathological lesions were produced in rats given 14 consecutive daily oral gavage doses of artelinic acid of 40 mg/kg/day or higher. In addition, drug-related hematological changes (increased mean reticulocyte counts) occurred in rats given 14 consecutive daily oral gavage doses of artelinic acid of 10 mg/kg/day or higher. The histopathological lesions observed in rats given intramuscular arteether were consistent with the previously reported neurotoxicity of this compound. Thus, from the results of this study information was obtained on a "no effect" dose for the production of neurotoxicity in rats given artelinic acid. A "no effect" dose for the observed hematological changes was not determined. These results were obtained using a limited number of rats per dose group. It is anticipated that confirmation of a "no effect" dose, delineation of target organ toxicity, and determination of the reversibility of any artelinic acid-induced toxicity will be obtained during the definitive study which is scheduled to be conducted during the upcoming contract year.

During the preliminary evaluation of the pharmacodynamic effects of artelinic acid in dogs, no drug-related mortality was observed at doses of 320 mg/kg/day and lower; with the exception of sporadic diarrhea, which was observed at all artelinic acid dose levels administered (20-320 mg/kg/day), no clinical signs of toxicity or body weight losses were observed for dogs given 20, 40, or 80 mg/kg/day of artelinic acid. Drug-related neuropathological lesions were not observed in animals given 14 consecutive daily oral gavage doses of artelinic acid of 20 mg/kg/day or higher; drug-related histopathological lesions were also not observed in non-brain tissues obtained from these same animals. Drug-related hematological changes were observed in dogs given 14 consecutive daily oral gavage doses of artelinic acid of 20 mg/kg/day and higher. From these data, a highest "no effect" dose of artelinic acid for the production of neurohistopathological and other histopathological toxicity in dogs was not established; in addition, a 'no effect' dose for the production of hematological changes was not established. The results did indicate, however, that the dose of artelinic acid that effected hematological changes was at least an order of magnitude lower than the dose that effected histopathological changes. It is anticipated that confirmation of a "no effect" dose, delineation of target organ toxicity, and determination of the reversibility of any artelinic acid-induced toxicity will be obtained during the definitive phase of this study.

6.0 REFERENCES

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Table 1

Effect of Artelincic Acid on Rats after Oral Administration for 14 Days

Summary Body Weights (grams): Males

Group Sex	Day numbers relative to Start Date													
	-2	-1	1	2	3	4	5	6	7	8	9	10	11	
VM	Mean	256.40	265.90	271.75	278.30	286.75	291.70	297.85	305.90	310.85	314.50	321.90	328.95	
	S.D.	
	N	2	2	2	2	2	2	2	2	2	2	2	2	
AM	Mean	249.90	264.00	261.60	262.85	269.85	277.18	283.90	288.70	295.30	300.43	305.93	311.85	
	S.D.	.	6.48	11.94	14.44	11.94	9.99	6.77	6.03	6.58	4.89	5.81	7.84	
	N	2	4	4	4	4	4	4	4	4	4	4	4	
BH	Mean	256.80	258.93	267.75	271.35	279.18	286.35	292.80	299.10	309.68	314.45	320.43	330.90	
	S.D.	.	10.64	12.88	11.34	14.62	14.42	13.44	11.05	15.37	14.09	15.93	16.92	
	N	2	4	4	4	4	4	4	4	4	4	4	4	
CM	Mean	265.95	251.50	271.70	274.80	281.25	286.03	289.85	294.30	300.45	307.63	309.43	314.63	
	S.D.	.	.	12.10	12.27	14.12	9.65	12.35	11.48	14.91	9.84	13.28	14.77	
	N	2	2	4	4	4	4	4	4	4	4	4	4	
DM	Mean	258.15	263.83	274.05	273.38	278.85	283.00	284.25	288.83	296.80	302.30	295.93	303.50	
	S.D.	.	6.34	5.73	8.71	9.08	7.18	7.66	8.40	10.34	9.78	15.48	10.55	
	N	2	4	4	4	4	4	4	4	4	4	4	4	
EM	Mean	253.10	266.30	272.90	257.20	251.25	244.20	237.20	233.45	227.25	221.75	218.05	210.20	
	S.D.	
	N	2	2	2	2	2	2	2	2	2	2	2	2	
FM	Mean	.	260.35	270.95	278.75	283.70	289.45	297.05	300.25	290.95	312.75	316.95	316.80	
	S.D.	
	N	0	2	2	2	2	2	2	2	2	2	2	2	

(.) = Not applicable

a All animals in group dead prior to scheduled collection period.

Group V - 0 mg/kg/day

Group A - 10 mg/kg/day Artelincic Acid

Group C - 40 mg/kg/day Artelincic Acid

Group F - 12.5 mg/kg/day Arteether

Group B - 20 mg/kg/day Artelincic Acid

Group E - 320 mg/kg/day Artelincic Acid

Table 1 (continued)

Effect of Artelinic Acid on Rats after Oral Administration for 14 Days

Summary Body Weights (grams): Males

Group Sex	Day numbers relative to Start Date					
		12	13	14	21	
VM	Mean	333.20	337.15	345.95	357.85	
	S.D.	
	N	2	2	2	2	
AM	Mean	319.88	325.90	328.33	337.18	
	S.D.	10.14	9.88	8.19	8.32	
	N	4	4	4	4	
BM	Mean	332.10	348.38	350.63	366.30	
	S.D.	19.41	17.42	14.45	17.27	
	N	4	4	4	4	
CM	Mean	326.65	332.48	337.25	352.78	
	S.D.	18.17	17.96	18.86	22.12	
	N	4	4	4	4	
DM	Mean	310.25	317.85	325.40	345.00	
	S.D.	11.84	11.19	16.80	16.04	
	N	4	4	4	4	
EM	Mean	199.90	198.40	197.85	195.30	
	S.D.	
	N	2	2	2	2	
FM	Mean	331.35	332.10	333.50	344.30	
	S.D.	
	N	2	2	2	2	

(.) = Not applicable

^a All animals in group dead prior to scheduled collection period.

Group V - 0 mg/kg/day

Group C - 40 mg/kg/day Artelinic Acid

Group F - 12.5 mg/kg/day Arteether

Group A - 10 mg/kg/day Artelinic Acid

Group D - 80 mg/kg/day Artelinic Acid

Group B - 20 mg/kg/day Artelinic Acid

Group E - 320 mg/kg/day Artelinic Acid

Table 1 (continued)

Effect of Artelinic Acid on Rats after Oral Administration for 14 Days

Summary Body Weights (grams): Females

Group Sex	Day numbers relative to Start Date													
	-2	-1	1	2	3	4	5	6	7	8	9	10	11	
VF	Mean 205.60	216.20	215.35	220.50	225.75	228.70	227.35	229.10	237.50	240.85	239.95	241.65		
	S.D.	2	2	2	2	2	2	2	2	2	2	2	2	
	N	2	2	2	2	2	2	2	2	2	2	2	2	
AF	Mean 209.15	210.60	213.70	212.38	215.13	219.98	218.33	223.58	227.95	227.88	227.78	231.18		
	S.D.	9.48	6.36	9.26	10.86	9.02	6.70	11.40	9.54	6.51	9.25	10.15		
	N	4	4	4	4	4	4	4	4	4	4	4	4	
BF	Mean 203.50	211.93	213.18	214.03	215.23	217.83	217.68	215.73	219.08	223.10	218.25	220.20	223.85	
	S.D.	7.05	8.64	7.41	6.73	7.86	7.85	6.72	4.46	8.45	6.33	2.96	4.02	
	N	4	4	4	4	4	4	4	4	4	4	4	4	
CF	Mean 213.75	201.65	210.38	206.78	211.48	211.98	208.38	209.20	209.65	212.13	214.83	213.15	213.68	
	S.D.	2	3.97	9.51	10.80	10.66	5.74	11.49	15.56	6.54	11.12	9.68	6.88	
	N	2	4	4	4	4	4	4	4	4	4	4	4	
DF	Mean 207.65	211.13	211.15	206.53	205.85	196.23	195.20	192.48	201.08	202.30	196.73	201.45	201.48	
	S.D.	4.91	6.51	6.79	10.21	5.13	9.61	9.28	6.98	12.80	14.06	11.69	14.86	
	N	4	4	4	4	4	4	4	4	4	4	4	4	
EF	Mean 203.95	213.40	196.15	189.75	184.30	178.90	169.50	161.50	153.05	145.30	140.05	142.00		
	S.D.	2	2	2	2	2	2	2	2	2	2	2	2	
	N	2	2	2	2	2	2	2	2	2	2	2	2	
FF	Mean 209.45	218.05	211.95	215.65	215.65	215.65	215.95	210.20	215.20	218.55	218.15	216.85	220.45	
	S.D.	2	2	2	2	2	2	2	2	2	2	2	2	
	N	2	2	2	2	2	2	2	2	2	2	2	2	

(.) = Not applicable
 a All animals in group dead prior to scheduled collection period.
 Group V - 0 mg/kg/day Artelinic Acid
 Group C - 40 mg/kg/day Artelinic Acid
 Group F - 12.5 mg/kg/day Arteether
 Group B - 20 mg/kg/day Artelinic Acid
 Group E - 320 mg/kg/day Artelinic Acid

Table 1 (continued)

Effect of Artelinic Acid on Rats after Oral Administration for 14 Days

Summary Body Weights (grams): Females

Group Sex	Day numbers relative to Start Date			
	12	13	14	21
VF	Mean	248.05	250.75	248.25
	S.D.	.	.	241.85
	N	2	2	2
AF	Mean	230.93	233.50	233.13
	S.D.	5.63	8.25	10.51
	N	4	4	4
BF	Mean	225.78	224.68	225.60
	S.D.	6.27	5.21	5.11
	N	4	4	4
CF	Mean	218.08	221.38	222.38
	S.D.	11.93	7.57	11.11
	N	4	4	4
DF	Mean	202.98	205.33	209.78
	S.D.	14.20	10.13	10.51
	N	4	4	4
EF	Mean	a		
	S.D.			
	N			
FF	Mean	224.40	225.40	224.05
	S.D.	.	.	215.30
	N	2	2	2

(.) = Not applicable

^a All animals in group dead prior to scheduled collection period.

Group V - 0 mg/kg/day

Group C - 40 mg/kg/day Artelinic Acid

Group F - 12.5 mg/kg/day Arteether

Group A - 10 mg/kg/day Artelinic Acid

Group D - 80 mg/kg/day Artelinic Acid

Group B - 20 mg/kg/day Artelinic Acid

Group E - 320 mg/kg/day Artelinic Acid

Table 2
Effect of Artelinic Acid on Rats after
Oral Administration for 14 Days

SUMMARY HEMATOLOGY REPORT
PERIOD: Day 21

STUDY ID: 9610.03.01

SEX: MALE

ANALYSIS OF VARIANCE FOLLOWED BY DUNNETT'S PROCEDURE

TEST(s):	WBC	RBC	HGB	HCT
UNITS:	thds/mm ³	mil/mm ³	g/dL	%
Group: VM : 0 mg/kg/day				
MEAN	13.43	8.04	15.8	48.1
SD	2.708	0.389	0.42	2.40
N	2	2	2	2
Group: AM : 10 mg/kg/day Artelinic Acid				
MEAN	10.07	7.57	14.7	44.7
SD	2.103	1.027	1.07	4.00
N	4	4	4	4
Group: BM : 20 mg/kg/day Artelinic Acid				
MEAN	14.31	7.67	15.9	49.4
SD	1.562	0.275	0.65	1.81
N	4	4	4	4
Group: CM : 40 mg/kg/day Artelinic Acid				
MEAN	8.95	7.88	15.1	47.5
SD	1.888	0.672	0.14	0.28
N	2	2	2	2
Group: DM : 80 mg/kg/day Artelinic Acid				
MEAN	9.46	6.88	14.8	45.2
SD	0.997	0.279	0.51	2.29
N	3	3	3	3
Group: EM : 320 mg/kg/day Artelinic Acid				
MEAN	9.01	7.37	14.6	45.3
SD	0.467	0.332	0.07	1.20
N	2	2	2	2
Group: FM : 12.5 mg/kg/day Arteether				
MEAN	12.62	7.64	14.5	45.2
SD	2.425	0.262	0.99	4.03
N	2	2	2	2

12-OCT-1999

Table 2 (continued)

Effect of Artelinic Acid on Rats after
Oral Administration for 14 Days

SUMMARY HEMATOLOGY REPORT
PERIOD: Day 21

STUDY ID: 9610.03.01

SEX: MALE

ANALYSIS OF VARIANCE FOLLOWED BY DUNNETT'S PROCEDURE

TEST(s):	MCV	MCH	MCHC	PLT	RETIC
UNITS:	fL	pg	g/dL	thds/mm ³	10**5/mm ³
Group: VM : 0 mg/kg/day					
MEAN	59.8	19.6	32.8	1110	2.7
SD	0.14	0.42	0.85	66.5	0.35
N	2	2	2	2	2
Group: AM : 10 mg/kg/day Artelinic Acid					
MEAN	59.3	19.5	32.9	1058	2.7
SD	3.11	1.68	1.13	129.1	0.34
N	4	4	4	4	4
Group: BM : 20 mg/kg/day Artelinic Acid					
MEAN	64.4	20.7	32.2	1194	4.8
SD	1.07	0.63	0.56	107.2	1.63
N	4	4	4	4	4
Group: CM : 40 mg/kg/day Artelinic Acid					
MEAN	60.6	19.3	31.8	1246	5.1
SD	4.74	1.48	0.07	7.8	0.14
N	2	2	2	2	2
Group: DM : 80 mg/kg/day Artelinic Acid					
MEAN	65.8	21.4	32.7	1181	5.2
SD	4.19	0.15	2.01	115.7	1.95
N	3	3	3	3	3
Group: EM : 320 mg/kg/day Artelinic Acid					
MEAN	61.4	19.8	32.2	1048	8.6**
SD	1.13	0.92	0.92	347.9	0.28
N	2	2	2	2	2
Group: FM : 12.5 mg/kg/day Arteether					
MEAN	59.1	19.1	32.2	1056	2.9
SD	3.18	0.64	0.71	143.5	0.49
N	2	2	2	2	2

**-Significant Difference from Control P < .01

07-OCT-1999

Table 2 (continued)

Effect of Artelinic Acid on Rats after
Oral Administration for 14 Days

SUMMARY HEMATOLOGY REPORT
PERIOD: Day 21

STUDY ID: 9610.03.01

SEX: MALE

ANALYSIS OF VARIANCE FOLLOWED BY DUNNETT'S PROCEDURE

TEST(s):	NEUT ABS	LYMPH ABS	MONO ABS	EOS ABS	BASO ABS	LUC ABS
UNITS:	thds/mm ³	thds/mm ³	thds/mm ³	thds/mm ³	thds/mm ³	thds/mm ³

Group: VM : 0 mg/kg/day						
MEAN	0.91	12.08	0.14	0.06	0.10	0.16
SD	0.057	2.680	0.035	0.000	0.007	0.064
N	2	2	2	2	2	2
Group: AM : 10 mg/kg/day Artelinic Acid						
MEAN	1.21	8.44	0.17	0.07	0.05	0.13
SD	0.366	1.774	0.116	0.006	0.024	0.033
N	4	4	4	4	4	4
Group: BM : 20 mg/kg/day Artelinic Acid						
MEAN	1.57	12.21	0.18	0.07	0.10	0.19
SD	0.416	1.085	0.057	0.045	0.030	0.031
N	4	4	4	4	4	4
Group: CM : 40 mg/kg/day Artelinic Acid						
MEAN	0.98	7.66	0.13	0.03	0.04	0.12
SD	0.530	1.273	0.049	0.000	0.014	0.021
N	2	2	2	2	2	2
Group: DM : 80 mg/kg/day Artelinic Acid						
MEAN	0.84	8.36	0.09	0.06	0.03*	0.08
SD	0.172	0.876	0.023	0.006	0.010	0.023
N	3	3	3	3	3	3
Group: EM : 320 mg/kg/day Artelinic Acid						
MEAN	1.00	7.69	0.16	0.02	0.04	0.12
SD	0.467	0.085	0.078	0.007	0.007	0.007
N	2	2	2	2	2	2
Group: FM : 12.5 mg/kg/day Arteether						
MEAN	1.09	11.15	0.11	0.08	0.07	0.13
SD	0.000	2.369	0.000	0.014	0.007	0.028
N	2	2	2	2	2	2

*--Significant Difference from Control P < .05

04-OCT-1999

Table 2 (continued)

Effect of Artelinic Acid on Rats after
Oral Administration for 14 Days

SUMMARY HEMATOLOGY REPORT
PERIOD: Day 21

STUDY ID: 9610.03.01

SEX: FEMALE

ANALYSIS OF VARIANCE FOLLOWED BY DUNNETT'S PROCEDURE

TEST(s):	MCV	MCH	MCHC	PLT	RETIC
UNITS:	fL	pg	g/dL	thds/mm ³	10**5/mm ³
Group: VF : 0 mg/kg/day					
MEAN	56.8	18.4	32.4	1171	1.9
SD	0.14	0.57	0.92	224.9	0.14
N	2	2	2	2	2
Group: AF : 10 mg/kg/day Artelinic Acid					
MEAN	57.3	19.0	33.2	1040	3.0
SD	0.36	0.74	1.08	265.1	0.71
N	4	4	4	4	4
Group: BF : 20 mg/kg/day Artelinic Acid					
MEAN	58.8	19.2	32.6	1105	4.4
SD	1.18	0.44	0.17	213.9	0.78
N	4	4	4	4	4
Group: CF : 40 mg/kg/day Artelinic Acid					
MEAN	58.0	19.3	33.2	1202	4.3
SD	0.73	0.05	0.46	226.2	0.87
N	4	4	4	4	4
Group: DF : 80 mg/kg/day Artelinic Acid					
MEAN	62.1*	20.4	32.8	1081	6.0**
SD	3.35	1.50	0.97	52.2	2.11
N	4	4	4	4	4
Group: EF : 320 mg/kg/day Artelinic Acid					
MEAN	NA	NA	NA	NA	NA
SD	NA	NA	NA	NA	NA
N	0	0	0	0	0
Group: FF : 12.5 mg/kg/day Arteether					
MEAN	57.5	18.9	32.9	1036	2.2
SD	2.12	0.99	0.64	19.8	0.28
N	2	2	2	2	2

*-Significant Difference from Control P < .05 NA-Not Applicable

**-Significant Difference from Control P < .01

04-OCT-1999

Table 2 (continued)

**Effect of Artelinic Acid on Rats after
Oral Administration for 14 Days**

**SUMMARY HEMATOLOGY REPORT
PERIOD: Day 21**

STUDY ID: 9610.03.01

SEX: FEMALE

ANALYSIS OF VARIANCE FOLLOWED BY DUNNETT'S PROCEDURE

TEST(s):	WBC	RBC	HGB	HCT
UNITS:	thds/mm ³	mil/mm ³	g/dL	%
Group: VF : 0 mg/kg/day				
MEAN	6.96	7.70	14.2	43.8
SD	3.352	0.962	1.34	5.30
N	2	2	2	2
Group: AF : 10 mg/kg/day Artelinic Acid				
MEAN	8.49	6.87	13.0	39.3
SD	1.686	1.134	2.02	6.44
N	4	4	4	4
Group: BF : 20 mg/kg/day Artelinic Acid				
MEAN	10.44	7.85	15.0	46.1
SD	4.460	0.162	0.19	0.67
N	4	4	4	4
Group: CF : 40 mg/kg/day Artelinic Acid				
MEAN	7.81	7.30	14.1	42.3
SD	2.723	0.668	1.25	3.90
N	4	4	4	4
Group: DF : 80 mg/kg/day Artelinic Acid				
MEAN	10.03	6.46	13.1	40.2
SD	2.904	0.945	2.17	6.61
N	4	4	4	4
Group: EF : 320 mg/kg/day Artelinic Acid				
MEAN	NA	NA	NA	NA
SD	NA	NA	NA	NA
N	0	0	0	0
Group: FF : 12.5 mg/kg/day Arteether				
MEAN	9.18	6.98	13.3	40.3
SD	0.891	1.259	3.04	8.70
N	2	2	2	2

NA-Not Applicable

04-OCT-1999

Table 2 (continued)

Effect of Artelinic Acid on Rats after
Oral Administration for 14 Days

SUMMARY HEMATOLOGY REPORT
PERIOD: Day 21

STUDY ID: 9610.03.01

SEX: FEMALE

ANALYSIS OF VARIANCE FOLLOWED BY DUNNETT'S PROCEDURE

TEST(s):	NEUT ABS	LYMPH ABS	MONO ABS	EOS ABS	BASO ABS	LUC ABS
UNITS:	thds/mm ³	thds/mm ³	thds/mm ³	thds/mm ³	thds/mm ³	thds/mm ³

Group: VF : 0 mg/kg/day						
MEAN	0.74	5.87	0.14	0.08	0.03	0.12
SD	0.516	2.496	0.113	0.092	0.028	0.106
N	2	2	2	2	2	2
Group: AF : 10 mg/kg/day Artelinic Acid						
MEAN	1.20	6.95	0.09	0.12	0.04	0.10
SD	0.834	1.448	0.053	0.095	0.026	0.029
N	4	4	4	4	4	4
Group: BF : 20 mg/kg/day Artelinic Acid						
MEAN	0.96	9.02	0.15	0.07	0.06	0.19
SD	0.691	3.977	0.102	0.021	0.031	0.165
N	4	4	4	4	4	4
Group: CF : 40 mg/kg/day Artelinic Acid						
MEAN	1.56	5.76	0.20	0.06	0.03	0.20
SD	1.543	1.709	0.139	0.022	0.013	0.133
N	4	4	4	4	4	4
Group: DF : 80 mg/kg/day Artelinic Acid						
MEAN	0.98	8.59	0.18	0.06	0.05	0.18
SD	0.820	2.493	0.031	0.024	0.026	0.053
N	4	4	4	4	4	4
Group: EF : 320 mg/kg/day Artelinic Acid						
MEAN	NA	NA	NA	NA	NA	NA
SD	NA	NA	NA	NA	NA	NA
N	0	0	0	0	0	0
Group: FF : 12.5 mg/kg/day Arteether						
MEAN	1.22	7.70	0.08	0.08	0.05	0.07
SD	0.615	0.269	0.021	0.000	0.007	0.028
N	2	2	2	2	2	2

NA-Not Applicable

04-OCT-1999

Table 3

Effect of Artelinic Acid on Rats after
Oral Administration for 14 Days

SUMMARY CLINICAL CHEMISTRY REPORT
PERIOD: DAY 21

STUDY ID: 9610.03.01

SEX: MALE

ANALYSIS OF VARIANCE FOLLOWED BY DUNNETT'S PROCEDURE

TEST(s):	NA	K	CL	ALP	ALT	AST
UNITS:	mmol/L	mmol/L	mmol/L	U/L	U/L	U/L
Group: VM : 0 mg/kg/day						
MEAN	147	6.5	97	227	40	76
SD	1.4	0.21	2.8	24.7	12.0	7.8
N	2	2	2	2	2	2
Group: AM : 10 mg/kg/day Artelinic Acid						
MEAN	147	6.2	99	156	35	86
SD	0.5	0.62	2.2	35.0	7.0	9.3
N	4	4	4	4	4	4
Group: BM : 20 mg/kg/day Artelinic Acid						
MEAN	146	6.7	100	159	38	107
SD	0.8	0.47	0.8	36.8	2.9	18.1
N	4	4	4	4	4	4
Group: CM : 40 mg/kg/day Artelinic Acid						
MEAN	147	6.5	99	148	38	95
SD	1.3	0.29	2.4	31.3	8.4	10.8
N	4	4	4	4	4	4
Group: DM : 80 mg/kg/day Artelinic Acid						
MEAN	145	6.7	100	162	48	99
SD	1.4	0.60	1.8	65.1	7.7	18.0
N	4	4	4	4	4	4
Group: EM : 320 mg/kg/day Artelinic Acid						
MEAN	146	6.7	99	111	38	111
SD	0.0	0.57	0.7	72.1	7.1	21.9
N	2	2	2	2	2	2
Group: FM : 12.5 mg/kg/day Arteether						
MEAN	146	6.4	100	179	38	87
SD	0.0	0.49	0.7	43.8	7.8	6.4
N	2	2	2	2	2	2

04-OCT-1999

Table 3 (continued)

Effect of Artelinic Acid on Rats after
Oral Administration for 14 Days

SUMMARY CLINICAL CHEMISTRY REPORT
PERIOD: DAY 21

STUDY ID: 9610.03.01

SEX: MALE

ANALYSIS OF VARIANCE FOLLOWED BY DUNNETT'S PROCEDURE

TEST(s):	BUN	CREA	BUN/CRE	GLUC	TP	ALB
UNITS:	mg/dL	mg/dL	ratio	mg/dL	g/dL	g/dL
Group: VM : 0 mg/kg/day						
MEAN	23.3	0.6	42.3	89	7.0	4.9
SD	2.76	0.07	0.42	7.8	0.14	0.14
N	2	2	2	2	2	2
Group: AM : 10 mg/kg/day Artelinic Acid						
MEAN	16.7	0.6	31.2	87	6.6	4.8
SD	3.62	0.10	8.66	11.0	0.24	0.17
N	4	4	4	4	4	4
Group: BM : 20 mg/kg/day Artelinic Acid						
MEAN	18.3	0.6	31.8	85	6.6	4.8
SD	4.50	0.05	6.70	9.4	0.22	0.10
N	4	4	4	4	4	4
Group: CM : 40 mg/kg/day Artelinic Acid						
MEAN	16.9	0.6	29.2	71	6.6	4.8
SD	3.59	0.05	4.76	30.2	0.17	0.13
N	4	4	4	4	4	4
Group: DM : 80 mg/kg/day Artelinic Acid						
MEAN	18.4	0.6	33.7	92	6.5	4.7
SD	4.69	0.06	9.65	15.7	0.30	0.22
N	4	4	4	4	4	4
Group: EM : 320 mg/kg/day Artelinic Acid						
MEAN	25.1	0.6	45.5	122	6.6	4.8
SD	4.81	0.07	2.90	7.1	0.64	0.35
N	2	2	2	2	2	2
Group: FM : 12.5 mg/kg/day Arteether						
MEAN	21.6	0.5	43.6	108	6.6	4.8
SD	4.45	0.14	3.39	4.2	0.28	0.21
N	2	2	2	2	2	2

12-OCT-1999

Table 3 (continued)

Effect of Artelinic Acid on Rats after
Oral Administration for 14 Days

SUMMARY CLINICAL CHEMISTRY REPORT
PERIOD: DAY 21

STUDY ID: 9610.03.01

SEX: FEMALE

ANALYSIS OF VARIANCE FOLLOWED BY DUNNETT'S PROCEDURE

TEST(s):	NA	K	CL	ALP	ALT	AST
UNITS:	mmol/L	mmol/L	mmol/L	U/L	U/L	U/L

Group: VF : 0 mg/kg/day						
MEAN	145	5.9	98	108	30	83
SD	0.7	0.35	0.7	24.7	0.0	6.4
N	2	2	2	2	2	2
Group: AF : 10 mg/kg/day Artelinic Acid						
MEAN	145	5.8	99	78	30	80
SD	1.7	0.53	2.2	26.3	6.9	6.6
N	4	4	4	4	4	4
Group: BF : 20 mg/kg/day Artelinic Acid						
MEAN	145	6.0	99	77	32	104
SD	0.6	0.33	1.9	6.0	16.7	25.1
N	4	4	4	4	4	4
Group: CF : 40 mg/kg/day Artelinic Acid						
MEAN	145	6.0	99	83	33	91
SD	1.0	0.50	2.6	13.3	11.1	4.6
N	4	4	4	4	4	4
Group: DF : 80 mg/kg/day Artelinic Acid						
MEAN	145	6.3	102	110	30	108
SD	1.0	0.54	2.6	13.4	8.6	11.5
N	4	4	4	4	4	4
Group: EF : 320 mg/kg/day Artelinic Acid						
MEAN	NA	NA	NA	NA	NA	NA
SD	NA	NA	NA	NA	NA	NA
N	0	0	0	0	0	0
Group: FF : 12.5 mg/kg/day Arteether						
MEAN	145	5.9	100	94	28	97
SD	0.0	0.28	1.4	7.8	4.2	7.8
N	2	2	2	2	2	2

NA-Not Applicable

04-OCT-1999

Table 3 (continued)

Effect of Artelinic Acid on Rats after
Oral Administration for 14 Days

SUMMARY CLINICAL CHEMISTRY REPORT
PERIOD: DAY 21

STUDY ID: 9610.03.01

SEX: FEMALE

ANALYSIS OF VARIANCE FOLLOWED BY DUNNETT'S PROCEDURE

TEST(s):	BUN	CREA	BUN/CRE	GLUC	TP	ALB
UNITS:	mg/dL	mg/dL	ratio	mg/dL	g/dL	g/dL

Group: VF : 0 mg/kg/day						
MEAN	13.4	0.7	19.1	89	7.2	5.4
SD	2.97	0.00	4.24	6.4	0.35	0.28
N	2	2	2	2	2	2
Group: AF : 10 mg/kg/day Artelinic Acid						
MEAN	16.5	0.6	29.0	92	7.3	5.4
SD	3.12	0.05	7.37	14.0	0.30	0.29
N	4	4	4	4	4	4
Group: BF : 20 mg/kg/day Artelinic Acid						
MEAN	19.6	0.6	32.9	90	7.6	5.7
SD	2.99	0.12	1.59	2.6	0.36	0.28
N	4	4	4	4	4	4
Group: CF : 40 mg/kg/day Artelinic Acid						
MEAN	22.0	0.6	35.1	85	7.5	5.5
SD	4.55	0.13	2.83	11.1	0.48	0.36
N	4	4	4	4	4	4
Group: DF : 80 mg/kg/day Artelinic Acid						
MEAN	17.7	0.6	31.1	84	6.7	5.1
SD	6.87	0.05	12.83	20.5	0.22	0.19
N	4	4	4	4	4	4
Group: EF : 320 mg/kg/day Artelinic Acid						
MEAN	NA	NA	NA	NA	NA	NA
SD	NA	NA	NA	NA	NA	NA
N	0	0	0	0	0	0
Group: FF : 12.5 mg/kg/day Arteether						
MEAN	19.0	0.7	29.2	82	7.0	5.1
SD	2.40	0.07	0.57	9.2	0.14	0.21
N	2	2	2	2	2	2

NA-Not Applicable

04-OCT-1999

Table 4. Histopathological Findings for Rats given either Artelinic Acid or Arteether for 14 Days

Vehicle Control	
3M-9	Normal
3M-10	Normal
3F-31	Normal
3F-32	Normal
Arteether	
7M-21	Neuron degeneration +1,
7M-22	Neuron degeneration +2, gliosis +1
7F-43	Neuron degeneration +1, several suspicious cells
7F-44	Neuron degeneration +1
10 mg/kg Artelinic Acid	
6M-17	Normal
6M-18	Normal
6M-19	Normal
6M-20	Normal
6F-39	Trapezoid nucleus not identified
6F-40	Normal
6F-41	Normal
6F-42	Normal
20 mg/kg Artelinic Acid	
1M-1	Normal
1M-2	Normal
1M-3	Normal
1M-4	Normal
1F-23	Normal
1F-24	Normal
1F-25	Normal
1F-26	Normal
40 mg/kg Artelinic Acid	
4M-11	Incomplete perfusion, no lesions seen
4M-12	Normal
4M-13	Normal
4M-14	Normal
4F-33	Neuron degeneration, +1
4F-34	Normal
4F-35	Neuron degeneration +1
4F-36	Neuron degeneration +1
80 mg/kg Artelinic Acid	
2M-5	Neuron degeneration, +2
2M-6	Neuron degeneration, +2
2M-7	Neuron degeneration, +2
2M-8	Neuron degeneration, +2
2F-27	Neuron degeneration, +1
2F-28	Neuron degeneration, +2
2F-29	Neuron degeneration, +1
2F-30	Neuron degeneration, +1
320 mg/kg Artelinic Acid	
5M-15	Neuron degeneration, +4, multiple nuclei in hind brain
5M-16	Neuron degeneration, +4, multiple nuclei in hind brain
5F-37	Died on study, not perfused, possible neuron degeneration
5F-38	Died on study, not perfused

Table 5

Effect of Artelinic Acid on Dogs After Oral Administration for 14 Days

Individual Body Weights: Males

		Body Weights (kg) on Study Day													
Group Sex	Dose Level	Animal Number	-4	1	2	3	4	5	6	7	8				
VM	0 mg/kg/day	2598	5.9	5.9	5.7	5.7	5.8	5.7	5.8	5.7	5.8	5.8			
		2594	7.6	7.6	7.5	7.7	7.5	7.9	7.7	7.7	7.6	7.6			
VM	0 mg/kg/day	2598	6.1	6.0	5.9	6.1	6.1	6.1	NA	5.9					
		2594	7.8	7.6	7.5	7.9	7.7	8.0	7.8						
AM	20 mg/kg/day Artelinic Acid	2599	7.0	7.1	7.0	6.8	6.9	6.9	6.9	6.9	6.9	6.9			
		2602	6.7	6.5	6.5	6.4	6.3	6.4	6.2	6.3	6.3	6.3			
AM	20 mg/kg/day Artelinic Acid	2599	7.0	6.9	6.9	6.9	6.9	6.9	6.8	6.8	6.8				
		2602	6.6	6.5	6.4	6.3	6.3	6.6	NA	6.3	6.3	6.3			

NA = Not applicable

^a Animal dead prior to data collection period.

Table 5 (Continued)

Effect of Artelinic Acid on Dogs After Oral Administration for 14 Days

Individual Body Weights: Males

		Body Weights (kg) on Study Day																	
Group Sex	Dose Level	Animal Number	-4	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	21
BM	40 mg/kg/day Artelinic Acid	2600	6.7	6.7	6.6	6.5	6.5	6.5	6.5	6.4	6.5	6.5	6.4	6.4	6.4	6.4	6.5	NA	6.3
		2595	7.7	7.7	7.9	7.9	7.8	7.8	7.8	7.9	7.9	7.8	7.8	7.8	7.9	7.9	7.9	7.7	7.7
BM	40 mg/kg/day Artelinic Acid	2600	6.5	6.4	6.4	6.4	6.4	6.4	6.4	6.4	6.4	6.4	6.4	6.4	6.4	6.4	6.5	NA	6.3
		2695	7.8	7.8	7.8	7.9	7.8	7.8	7.8	7.9	7.9	7.9	7.8	7.8	7.9	7.9	7.9	7.7	a
CH	80 mg/kg/day Artelinic Acid	2604	6.1	6.0	6.2	5.9	6.2	5.9	6.2	5.9	6.2	5.9	6.2	5.9	6.2	6.2	5.9	5.8	5.7
		2596	6.8	6.6	6.5	6.5	6.6	6.6	6.5	6.4	6.6	6.5	6.5	6.5	6.6	6.6	6.4	6.4	6.6
CH	80 mg/kg/day Artelinic Acid	2604	6.1	5.8	5.7	5.8	5.6	5.9	5.7	5.8	5.7	5.8	5.6	5.9	5.7	5.6	5.9	NA	5.6
		2596	6.5	6.4	6.3	6.5	6.3	6.3	6.4	6.3	6.5	6.3	6.3	6.4	6.4	6.4	6.4	6.4	a

NA = Not applicable
 a Animal dead prior to data collection period.

Table 5 (Continued)

Effect of Artelinic Acid on Dogs After Oral Administration for 14 Days

Individual Body Weights: Males

		Body Weights (kg) on Study Day													
Group Sex	Dose Level	Animal Number	-4	1	2	3	4	5	6	7	8				
DH	320 mg/kg/day Artelinic Acid	2605	6.3	6.1	5.9	5.8	5.9	5.6	5.7	5.6	5.5				
		2601	7.2	7.0	7.0	6.7	6.8	6.4	6.5	6.3	6.3				
DH	320 mg/kg/day Artelinic Acid	2605	5.4	5.7	5.6	5.5	5.4	5.5	NA	5.6					
		2601	6.3	6.3	6.2	6.2	6.1	6.3	5.9	5.9					
EM	20 mg/kg/day Arteether	2597	7.8	7.8	7.6	7.6	7.5	7.5	7.5	7.5	7.4				
		2603	6.6	6.5	6.4	6.5	6.6	6.4	6.6	6.6	6.7				
EM	20 mg/kg/day Arteether	2597	7.4	7.2	7.1	7.1	7.2	7.2	7.0	7.0	7.4				
		2603	6.9	6.8	6.7	6.9	6.9	7.1	NA	NA	6.8				

NA = Not applicable

^a Animal dead prior to data collection period.

Table 5 (Continued)

Effect of Artelinic Acid on Dogs After Oral Administration for 14 Days

Individual Body Weights: Females

		Body Weight (kg) on Study Day															
Group Sex	Dose Level	Animal Number	-5	1	2	3	4	5	6	7	8						
VF	0 mg/kg/day	2612	5.3	5.1	5.1	5.2	5.1	5.1	5.0	5.1	5.1	5.1	5.0	5.1	5.1	5.1	
		2611	6.4	6.2	6.3	6.2	6.1	6.1	6.2	6.1	6.2	6.1	6.1	6.1	6.1	6.1	6.4
VF	0 mg/kg/day	2612	5.1	5.0	5.2	5.1	5.2	5.2	NA	5.2	5.1	5.1	NA	5.1	5.1	5.1	
		2611	6.1	6.2	6.2	6.1	6.1	6.1	6.3	6.0	6.3	6.1	6.0	6.0	6.1	6.1	a
AF	20 mg/kg/day Artelinic Acid	2618	5.6	5.3	5.4	5.3	5.3	5.2	5.5	5.2	5.3	5.2	5.5	5.5	5.5	5.4	
		2614	5.8	5.6	5.7	5.6	5.5	5.7	5.7	5.6	5.7	5.6	5.6	5.6	5.6	5.7	5.7
AF	20 mg/kg/day Artelinic Acid	2618	5.3	5.2	5.4	5.2	5.2	5.3	NA	5.3	5.2	5.2	NA	5.7	5.7	5.7	
		2614	5.8	5.6	5.7	5.6	5.7	5.6	5.6	5.6	5.7	5.6	5.6	5.6	5.6	5.6	a

NA = Not applicable

a Animal dead prior to data collection period.

Table 5 (Continued)

Effect of Artelinic Acid on Dogs After Oral Administration for 14 Days

Individual Body Weights: Females

Body Weights (kg) on Study Day

Group Sex	Dose Level	Animal Number	-5	1	2	3	4	5	6	7	8
BF	40 mg/kg day Artelinic Acid	2616	6.2	6.2	6.2	6.4	6.1	6.1	6.1	6.2	6.2
		2606	5.8	5.6	5.5	5.5	5.3	5.6	5.5	5.6	5.7
BF	40 mg/kg/day Artelinic Acid	2616	6.1	6.1	6.2	6.1	6.3	6.3	NA	6.4	
		2606	5.4	5.5	5.5	5.3	5.5	5.5	5.2	a	
CF	80 mg/kg/day Artelinic Acid	2609	5.4	5.3	5.3	5.3	5.2	5.2	5.2	5.2	5.2
		2587	7.3	7.3	7.2	7.3	7.2	7.2	7.2	7.3	7.2
CF	80 mg/kg/day Artelinic Acid	2609	5.2	5.1	5.2	5.1	5.3	5.2	NA	5.4	
		2587	7.1	7.1	7.3	7.2	7.2	7.0	7.0	a	

NA = Not applicable

^a Animal dead prior to data collection period.

Table 5 (Continued)
 Effect of Artelinic Acid on Dogs After Oral Administration for 14 Days

Individual Body Weights: Females

		Body Weights (kg) on Study Day															
Group Sex	Dose Level	Animal Number	-5	1	2	3	4	5	6	7	8						
DF	320 mg/kg/day Artelinic Acid	2608	5.8	5.9	5.7	5.8	5.6	5.5	5.4	5.3	5.2						
		2607	5.8	6.0	5.7	5.6	5.6	^a									
DF	320 mg/kg/day Artelinic Acid	2608	5.1	5.0	5.0	4.8	4.7	4.6	4.5	5.1							
		2607															
EF	20 mg/kg/day Arteether	2615	5.6	5.4	5.6	5.6	5.5	5.4	5.5	5.3	5.4						
		2610	6.8	6.6	6.7	6.7	6.5	6.5	6.5	6.5	6.5	6.5					
EF	20 mg/kg/day Arteether	2615	5.2	5.0	4.9	4.8	4.9	4.9	4.6	4.6	4.6						
		2610	6.3	6.1	5.8	5.6	5.4	5.3	5.2	5.2	5.2						

NA = Not applicable
^a Animal dead prior to data collection period.

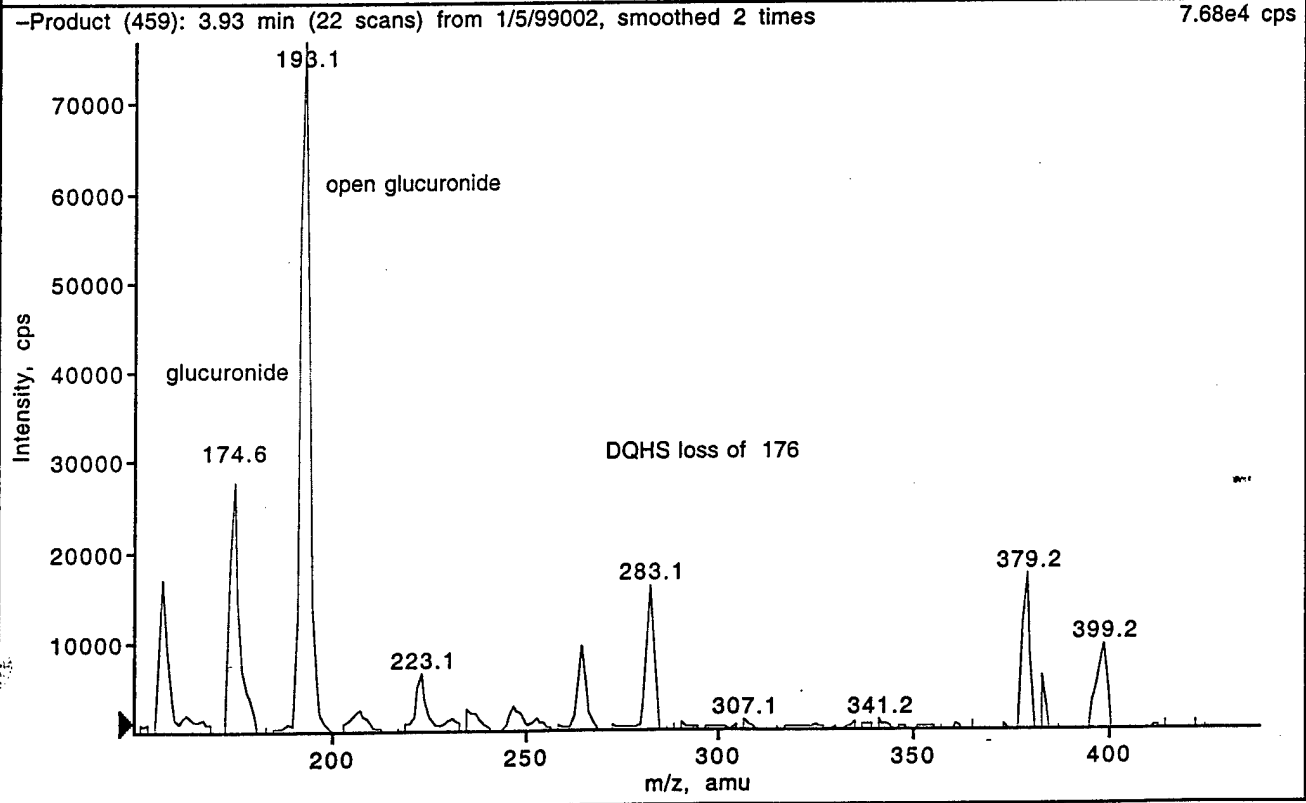
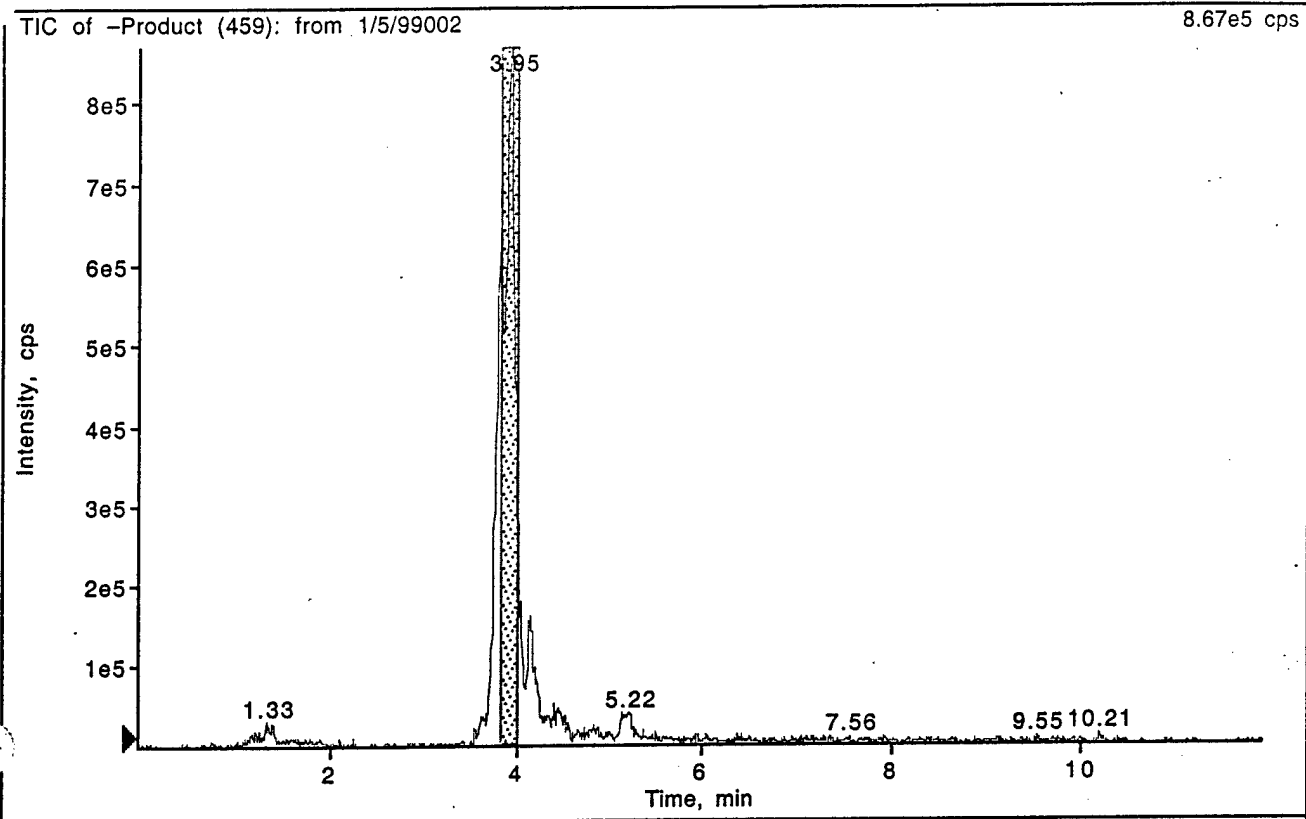


Figure 1. LC/MS/MS selected ion chromatogram of urine collected from a dog given artelinic acid (top panel) and the mass spectrum of the metabolite tentatively identified as DQHS glucuronide (bottom panel).

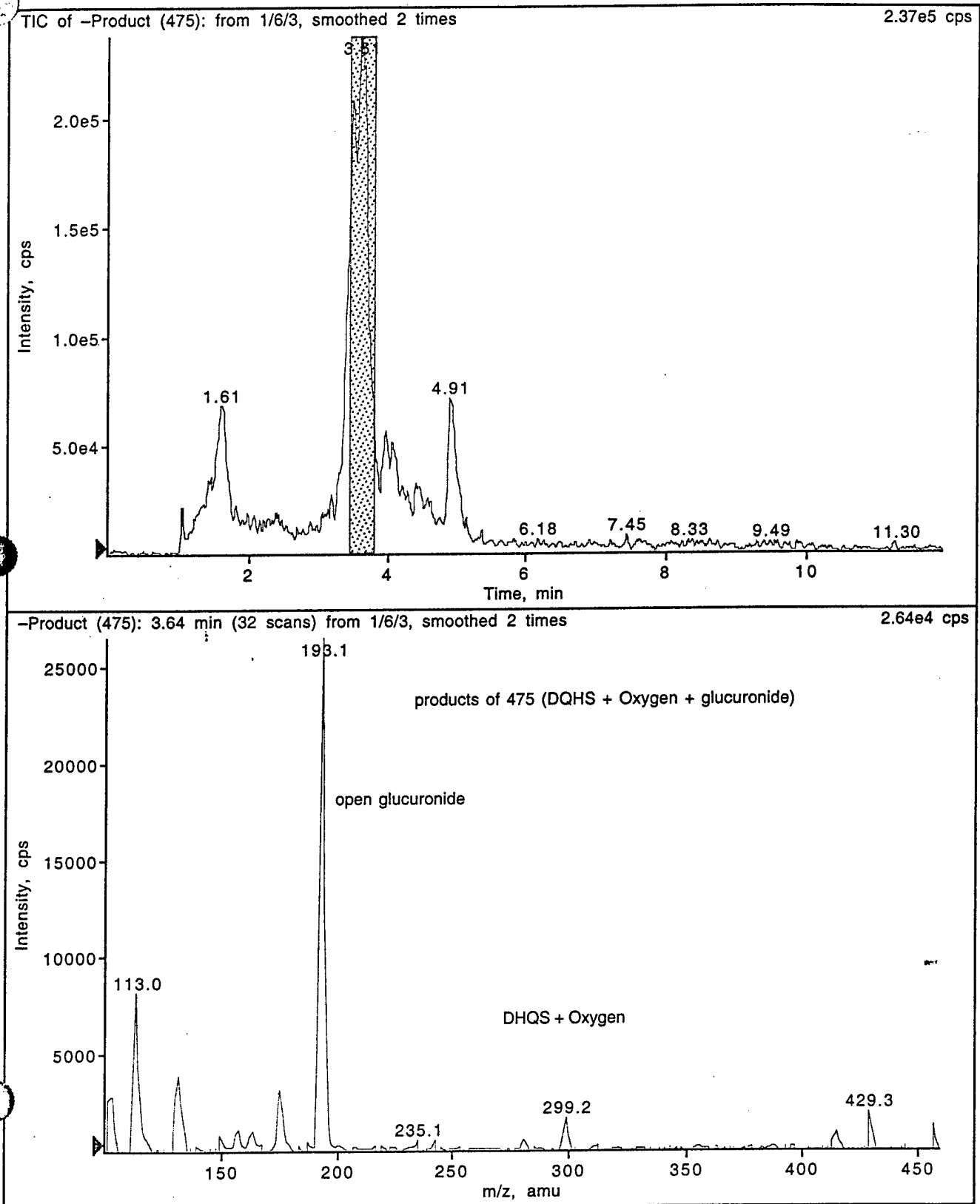


Figure 2. LC/MS/MS selected ion chromatogram of urine collected from a dog given artelinic acid (top panel) and the mass spectrum of the metabolite tentatively identified as a glucuronide of hydroxy DQHS (bottom panel).

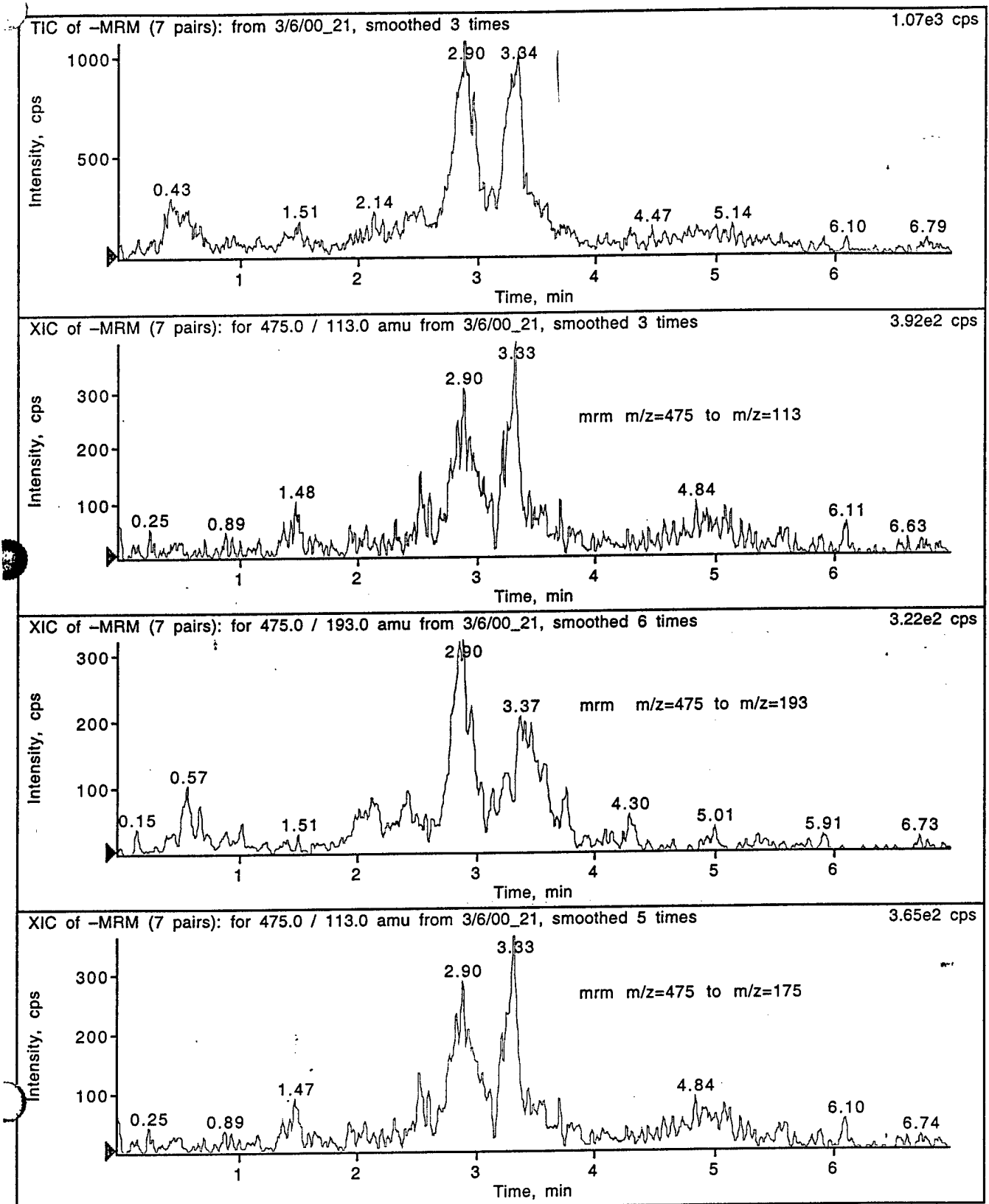


Figure 3. LC/MS/MS chromatogram of the urinary metabolite of artelinic acid tentatively identified as a glucuronide of hydroxy DQHS.

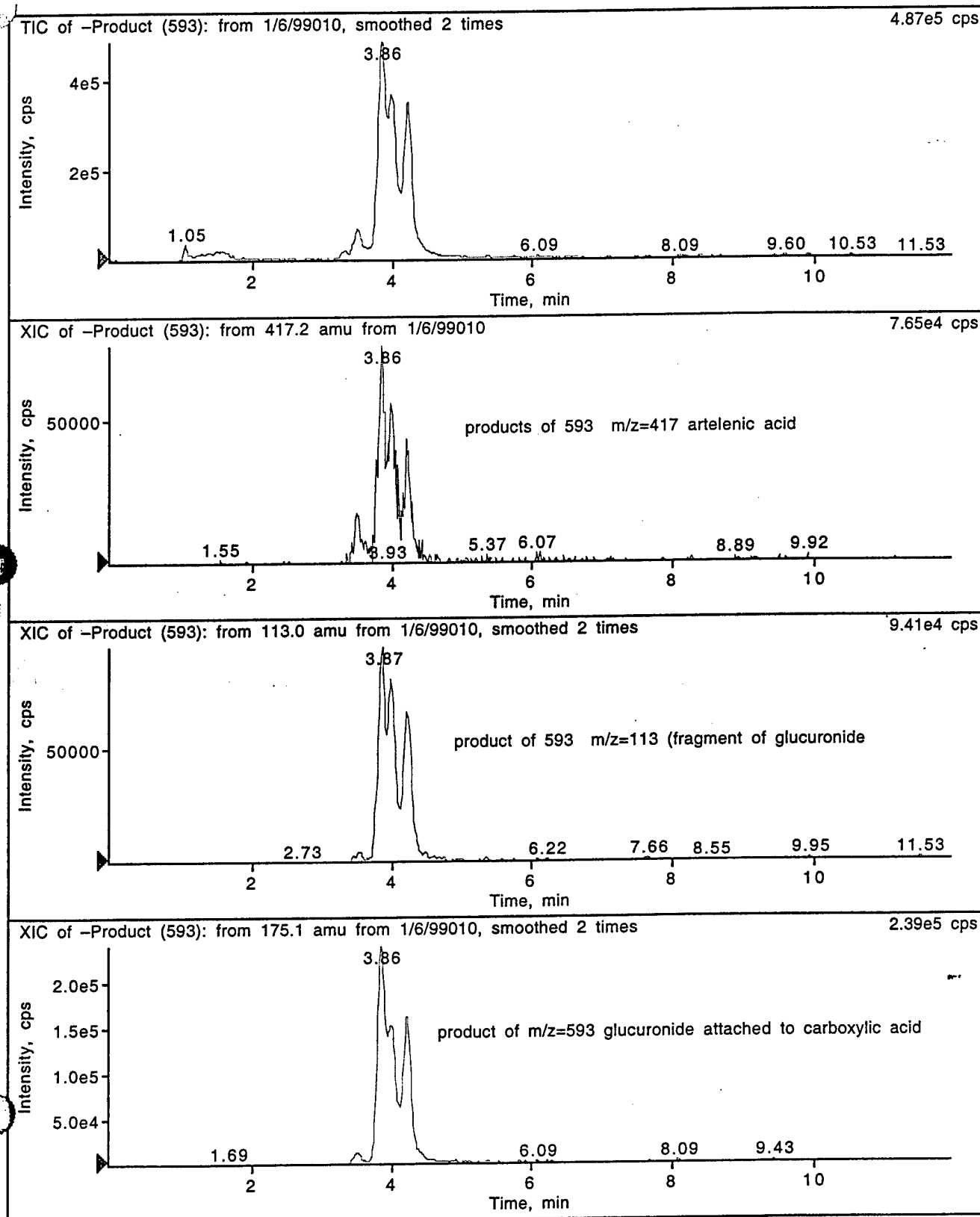


Figure 4. LC/MC/MS chromatogram of the urinary metabolite of artelinic acid tentatively identified as a glucuronide of artelinic acid.

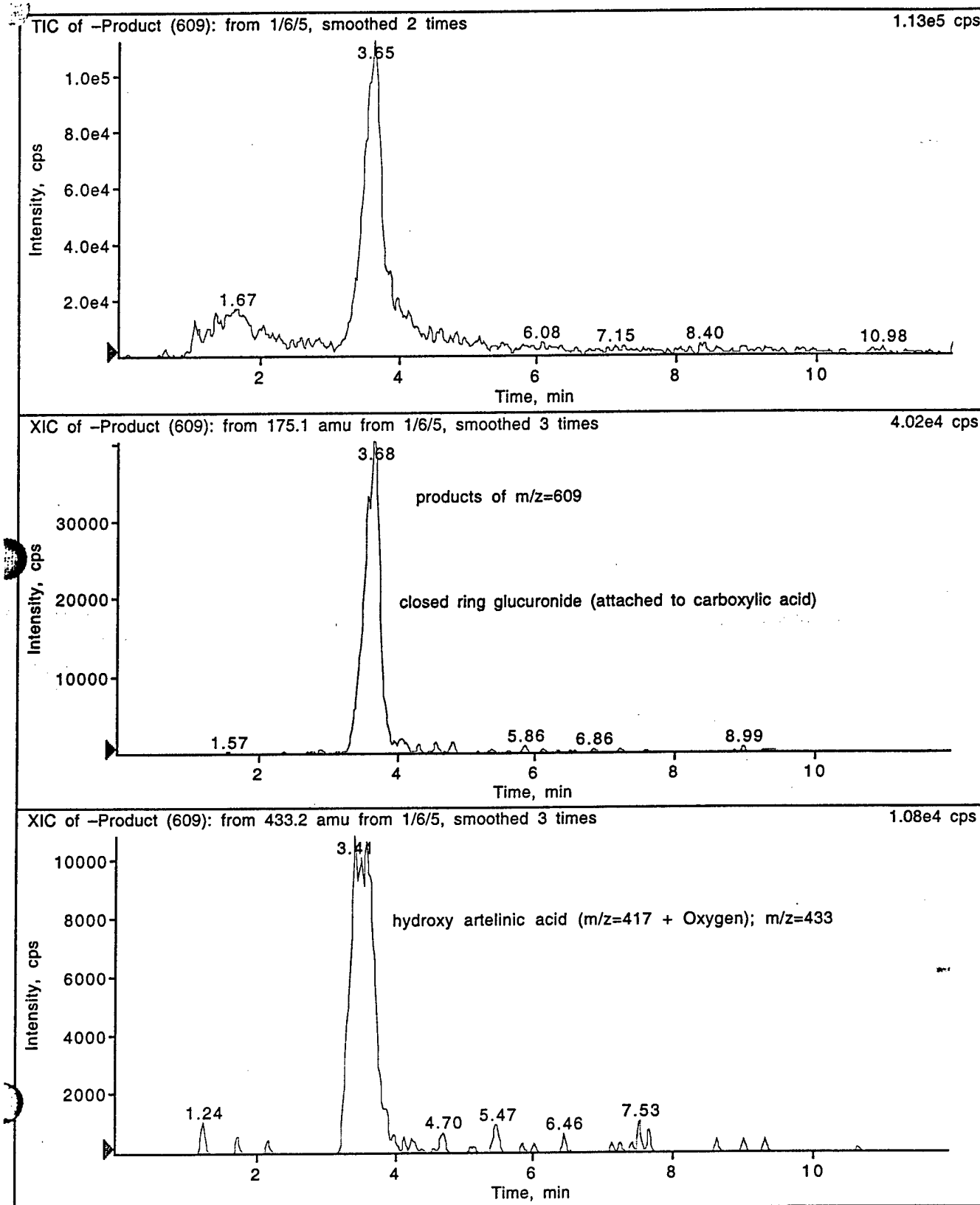


Figure 5. LC/MS/MS chromatogram of the urinary metabolite of artelinic acid tentatively identified as a glucuronide of hydroxy artelinic acid.