

**CENTER FOR DRUG EVALUATION AND  
RESEARCH**

*APPLICATION NUMBER:*  
**NDA 21-799**

**PHARMACOLOGY REVIEW(S)**



DEPARTMENT OF HEALTH AND HUMAN SERVICES  
PUBLIC HEALTH SERVICE  
FOOD AND DRUG ADMINISTRATION  
CENTER FOR DRUG EVALUATION AND RESEARCH

## PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER: 21-799  
SERIAL NUMBER: 000  
DATE RECEIVED BY CENTER: 10/13/04  
PRODUCT: Quinine Sulfate  
INTENDED CLINICAL POPULATION: treatment of malaria (*P. falciparum*)  
SPONSOR: Mutual Pharmaceutical Co., Inc.  
DOCUMENTS REVIEWED: electronic submission  
REVIEW DIVISION: Division of Special Pathogen and  
Immunologic Drug Products (HFD-590)  
PHARM/TOX REVIEWER: Steven Kunder, Ph.D.  
PHARM/TOX SUPERVISOR: Robert Osterberg, Ph.D.  
DIVISION DIRECTOR: Renata Albrecht, MD  
PROJECT MANAGER: Kristen Miller, PharmD

Date of review submission to Division File System (DFS):

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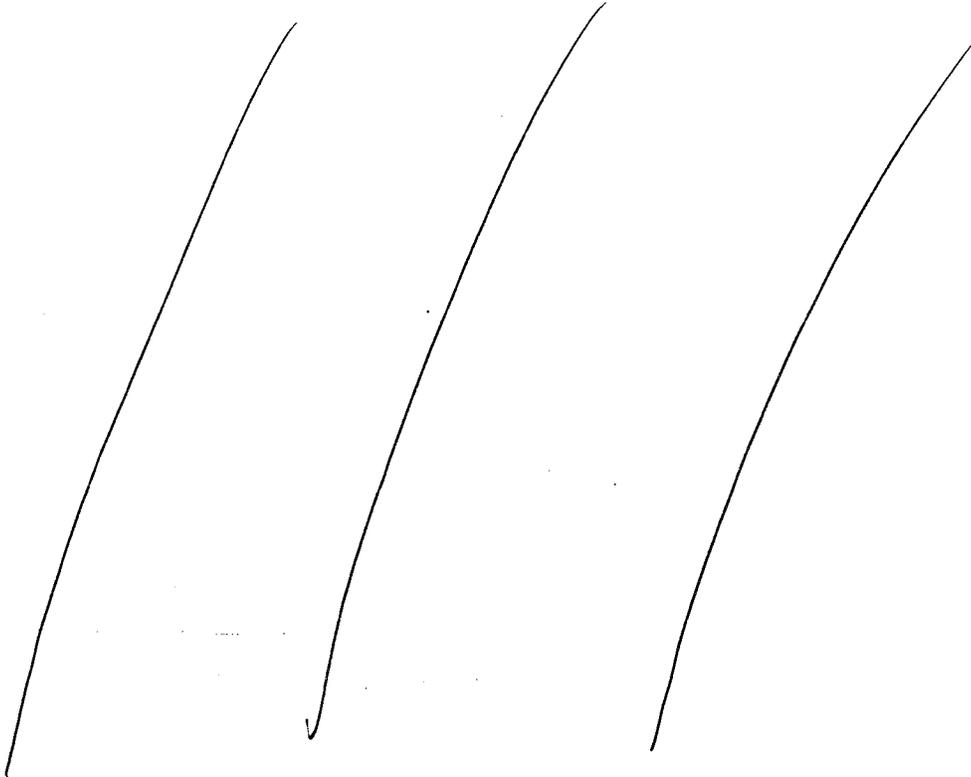
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***EXECUTIVE SUMMARY*****I. Recommendations****A. Recommendation on approvability**

This submission is acceptable with regard to pharmacology and toxicology issues. The main deficiencies, lack of nonrodent toxicity studies and male fertility studies, are compensated by the abundance of human clinical toxicity data from years of clinical usage experience.

**B. Recommendation for nonclinical studies:**

No further nonclinical studies are recommended

**C. Recommendations on labeling:**

## II. Summary of nonclinical findings

### A. Brief overview of nonclinical findings

#### Nonclinical Pharmacology

Published nonclinical studies related to the safety pharmacology / secondary pharmacodynamics of quinine were provided. Cardiovascular effects of quinine include QT prolongation, antiarrhythmic activity in animal models, and lowered blood pressure, similar to those of quinidine, but quinine is less potent and effects are seen at concentrations higher than those achieved clinically. Other effects include those on skeletal muscle (decreased response to nerve impulse stimulation), the auditory system (ototoxicity by toxic effects on hair cells), and glucose control (hypoglycemia).

#### Toxicity

Repeated dose toxicity studies were performed in rats for 13 weeks (oral) and 15 months (drinking water). The chronic toxicity of quinine sulfate (15 months) included mortality and adverse liver effects. The liver toxicities include periportal glycogen depletion in all lobular areas and mild periportal fibrosis, small areas of fibrosis of the bile duct, Kupffer cells with varying degrees of lipid accumulation in the form of large cytoplasmic droplets, abundant binucleate hepatocytes, and increased numbers of lysosomes. There was no hyperplasia or evidence of liver necrosis in the treated animals. The 15-month study was conducted with quinine sulfate in drinking water. The estimated dose was 100 mg/kg/day, based on an assumed water consumption of 20 mL/rat/day.

Subchronic toxicity studies (13 weeks) in rats showed tolerance up to 120 mg/kg/day without significant liver effects or mortality. Non-rodent toxicity data was not available. The extensive experience of human use for more than one hundred years and the available toxicological profile in rats negates the need for toxicity information from non-rodent species.

#### Reproductive toxicity

Teratogenic effects were observed in rabbits, guinea pigs, chinchilla, and dog, and not observed in mice, and monkeys; results were equivocal in rats. Teratogenic effects were observed in rabbits (death *in utero*, degenerated auditory nerve and spiral ganglion, CNS anomalies such as anencephaly and microcephaly), guinea pigs (hemorrhage and mitochondrial change in cochlea), and chinchillas (death and growth suppression *in utero*, CNS anomalies such as anencephaly and microcephaly). There were no teratogenic findings in mice and monkeys. Embryo-fetal deaths or toxicities were observed in mice, rabbits, chinchillas and dogs, but not in rats or monkeys. The lowest observed adverse effect levels (LOAELs) for teratogenicity were approximately 200 mg/kg/day or 1600 mg/m<sup>2</sup>/day (guinea pig), 130 mg/kg/day i.m. or 1560 mg/m<sup>2</sup>/day (rabbit), and 150 mg/kg/day s.c. (chinchilla). The LOAELs were more than 1-fold of the maximum recommended human dose of 32.4 mg/kg/day (or 1199 mg/m<sup>2</sup>/day) for a 60-kg patient, on a mg/m<sup>2</sup> basis. The no-observed adverse effect levels (NOAELs) for reproductive toxicity were 500 mg/kg/day or 1500 mg/m<sup>2</sup>/day (mouse), 300 mg/kg/day p.o. or 1800 mg/m<sup>2</sup>/day (rat), 50 mg/kg/day, i.m. or 1000 mg/m<sup>2</sup>/day (dog), and 200 mg/kg/day p.o. or 2400 mg/m<sup>2</sup>/day (monkey). These NOAELs are approximately 1.3x(mouse), 0.8x (dog), 1.5x (rat) and 2.0x (monkey) of the maximum

recommended human dose of 32.4 mg/kg/day (or 1199 mg/m<sup>2</sup>/day) for a 60-kg patient, on a mg/m<sup>2</sup> basis.

In rats, no impairment of female fertility was observed at an estimated dose of 20 mg/kg/day (or 120 mg/m<sup>2</sup>/day) of quinine sulfate administered in drinking water for a period from 2 weeks prior to mating and continuing during gestation and post-natal development. Animal data on the effect of quinine on male fertility were not available. These NOAELs are approximately 1/10-fold (female fertility) of the recommended human dose of 32.4 mg/kg/day (or 1199 mg/m<sup>2</sup>/day) for a 60-kg patient, on a mg/m<sup>2</sup> basis.

Quinine sulfate was shown to cause adverse effects on prenatal and postnatal development in rats. In female rats receiving quinine sulfate in drinking water from 2 weeks prior to mating and continuing on during the entire pregnant and postnatal period, the pups showed impaired growth with lower body weights both at birth and during the lactation period, and delayed physical development of the teeth eruption and eyes opening during lactation period. The effects were observed at an estimated dose of 20 mg/kg/day or 120 mg/m<sup>2</sup>/day, which is approximately 1/10-fold of the maximum recommended human dose of 32.4 mg/kg/day or 1199 mg/m<sup>2</sup>/day for a 60-kg patient, on a mg/m<sup>2</sup> basis.

#### GENOTOXICITY.

Genotoxicity was seen in the *in vitro* bacterial reverse mutation assays with metabolic activation, the sister chromatid exchange assay and the chromosomal aberration assay .

In the *in vitro* bacterial reverse mutation assays in *Salmonella typhimurium*, quinine was demonstrated as positive in the presence of metabolic activation (strain TA98), but negative in the absence of metabolic activation whereas all other strains were negative either in the presence or absence of metabolic activation.

In C3H mice, the *in vivo* micronucleus assay was positive following an oral dose of 110 mg/kg. while nonpositive findings were obtained in NMRI male and female mice following intraperitoneal or oral administrations (0.5 mmole/kg or 199 mg/kg) or following an oral dose of 110 mg/kg in Chinese hamsters. The *in vivo* sister chromatid exchange (SCE) tests showed positive results in all strains of mice tested (NMRI, C3H and C57B1), but negative in Chinese hamsters. The *In vivo* chromosomal aberration assays were not positive in NMRI and C3H mice at oral doses of 110 mg/kg. There were non-positive genotoxicity findings in the sex-linked recessive lethal test performed in *Drosophila* at a concentration of 0.39• g/ml.

#### B. Pharmacologic activity

The primary pharmacologic activity of quinine is its antimicrobial activity against the blood schizont form of *P.falciparum*. Quinine sulfate acts primarily on the blood schizont form of *P.falciparum*; it is not gametocidal and has little effect on the sporozoite or pre-erythrocytic forms. Because of this limited spectrum of antimalarial activity, quinine is not used for malaria prophylaxis. The antimalarial mechanism of action is proposed *via* inhibition of the *P.falciparum* heme polymerase, which is required to convert heme to the non-toxic malaria pigment hemozoin. Quinine sulfate is highly concentrated in the *P. falciparum* acidic food vacuoles, the putative site of inhibition of

the heme polymerase. It is unclear whether direct toxicity of the accumulated heme alone or involves a heme-quinine complex provides the antimalarial activity of quinine

C. Nonclinical safety issues relevant to clinical use

Nonclinical toxicities including QT prolongation, ototoxicity, and reproductive toxicity (teratogenesis, fetotoxicity and delayed development) are relevant to acute clinical use for treatment of *P. falciparum* malaria, as confirmed by extensive clinical findings.

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## 2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

### 2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 21-799

Review number: 001

Sequence number/date/type of submission: 000/ Oct 13 2004 /

Information to sponsor: Yes ( ) No ( )

Sponsor and/or agent: Mutual Pharmaceuticals Co. Inc.

1100 Orthodox St.

Philadelphia, PA 19124

(215) 288-6500

Manufacturer for drug substance: \_\_\_\_\_

Reviewer name: Steven Kunder, Ph.D.

Division name: Special Pathogen and Immunologic Drug Products

HFD #: 590

Review completion date: Aug 5, 2005

#### Drug:

Trade name: Quinine sulfate

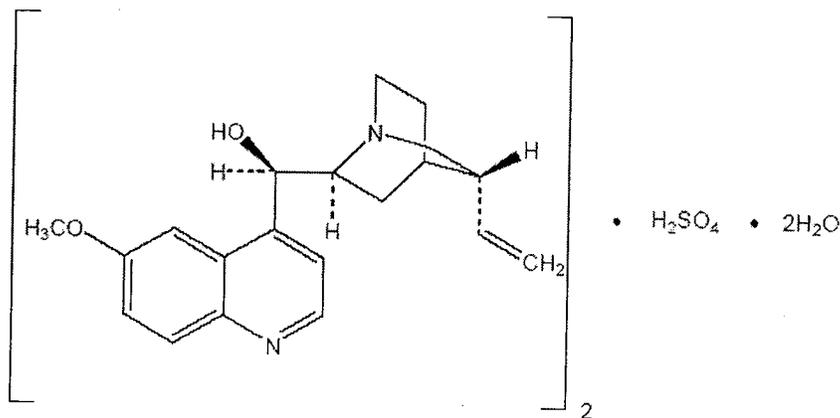
Generic name: quinine sulfate

Code name: none

Chemical name: Cinchonan-9-ol, 6'-methoxy-, (8 $\alpha$ ,9R)-, sulfate (2:1) (salt), dihydrate

Molecular formula/molecular weight:  $C_{20}H_{24}N_2O_2)_2 \cdot H_2SO_4 \cdot 2H_2O$  / 782.96

Structure:



**Relevant INDs/NDAs/DMFs:** IND 67, 012

**Drug class:** antimalarial

**Intended clinical population:** patients infected with *P. falciparum*

**Clinical formulation:** quinine sulfate in 324 mg tablet with corn starch, magnesium stearate, talc

**Route of administration:** oral tablet

**Disclaimer:** Tabular and graphical information are constructed by the reviewer unless cited otherwise.

**Data reliance :** Except as specifically identified below, all data and information discussed below and necessary for approval of NDA 21-799 are owned by Mutual Pharmaceutical Co., Inc. or are data for which Mutual Pharmaceutical Co., Inc has obtained a written right of reference. Any information or data necessary for approval of NDA 21-799 that Mutual Pharmaceutical Co., Inc does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as described in the drug's approved labeling. Any data or information described or referenced below from a previously approved application that Mutual Pharmaceutical Co., Inc does not own (or from FDA reviews or summaries of a previously approved application) is for descriptive purposes only and is not relied upon for approval of NDA 21-799.

As a 505(b)2 submission, all toxicology support was provided by references from the scientific literature. The sponsor included the following statement regarding the source of their references in support of their submission:

"The summary in this section is based on standard and comprehensive secondary references including the United States Pharmacopeia's Drug Information for the Healthcare Professional (2001), Martindale's Extra Pharmacopoeia (1996), and Goodman and Gilman's The Pharmacological Basis of Therapeutics (2001) unless otherwise referenced. Information is also excerpted from product labeling currently in use. In addition, searches of the worldwide literature were undertaken in order to identify all relevant sources of information. The searches were done using Dialog® by a trained research associate. On each occasion, the following databases were searched:

MEDLINE (1966 to present): Produced by the U.S. National Library of Medicine as a major source for biomedical literature.

EMBASE (1974 to present): Comprehensive index of the world's literature on human medicine and related disciplines (more comprehensive with respect to European studies than is MEDLINE).

JICST-Eplus (1985 to present): Covers Japanese and Asian literature in the fields of science, technology and medicine.

Biosis Previews (1969 to present): Worldwide coverage of research in the

biological and biomedical sciences (primarily identifies abstracts from meetings and symposia)

ToxFile (1965 to present): Covers the toxicological, pharmacological, biochemical, and physiological effects of drugs and other chemicals.

Registry of Toxic Effects of Chemical Substances (RTECS) (through 2001): Comprehensive database of basic toxicity information for chemical substances. “

**Studies reviewed within this submission:**

NDA 21-799 is a 505(b)2 application. The following is the listing of papers supplied by the sponsor in support of the application:

**5.6.1 Pharmacology**

Alvn G, Karlsson KK, Villn T. Reversible hearing impairment related to quinine blood concentrations in guinea pigs. *Life Sci.* 1989; 45:751-755.

Clark RB, Sanchez-Chapula J, Salinas-Stefanon E, Duff HJ, Giles WR. Quinidine-induced open channel block of K<sup>+</sup> current in rat ventricle. *Br. J. Pharmacol.* 1995; 115:335-343.

del Pozo BF, Perez-Vizcaino F, Villamor E, Zaragoza F, Tamargo J. Stereoselective effects of the enantiomers, quinidine and quinine, on depolarization- and agonist-mediated responses in rat isolated aorta. *Br. J. Pharmacol.* 1996; 117:105-110.

Hiatt EP. Effects of repeated oral doses of quinine and quinidine on the blood pressure and renal circulation of dogs with experimental neurogenic hypertension. *Am. J. Physiol.* 1948; 155:114-117.

Holloway PAH, Krishna S, White NJ. *Plasmodium berghei*: Lactic acidosis and hyposeycaemia in a rodent model of severe malaria; effects of glucose, quinine, and dichloroacetate. *Exp. Parasitol.* 1991; 72:123-133.

Jung TTK, Rhee C-K, Lee CS, Park Y-S, Choi D-C. Ototoxicity of salicylate, nonsteroidal anti-inflammatory drugs, and quinine. *Ototoxicity.* 1993; 26:791-810.

Jurkiewicz NK, Rodzinski JA, Colatsky TJ. Antiarrhythmic action of quinidine: relative importance of QT-prolongation vs use-dependence. *Circulation.* 1985; 71(Suppl II):II-225. (Abstract)

Klevans LR, Kelly RJ, Kovacs JL. Comparison of the antiarrhythmic activity of quinidine and quinine. *Arch. Int. Pharmacodyn.* 1977; 227:57-68.

Lee CS, Heinrich J, Jung TTK, Miller SK. Quinine-induced ototoxicity: Alterations in cochlear blood flow. *Otolaryngol. Head Neck Surg.* 1992; 107:233. (Abstract)

Mecca TE, Elam JT, Nash CB, Caldwell RW. (-Adrenergic blocking properties of quinine HCl. *Eur. J. Pharmacol.* 1980; 63:159-166.

Michel D, Wegener JW, Nawrath H. Effects of quinine and quinidine on the transient outward and on the L-type  $Ca^{2+}$  current in rat ventricular cardiomyocytes. *Pharmacol.* 2002; 65:187-192.

Okitolanda W, Pottier A-M, Henquin J-C. Glucose homeostasis in rats treated acutely and chronically with quinine. *Eur. J. Pharmacol.* 1986; 132:179-185.

Sanchez-Chapula JA, Ferrer T, Navarro-Polanco RA, Sanguinetti MC. Voltage-Dependent Profile Of Human *ether-a-go-go*-related gene channel block is influenced by a single residue in the S6 transmembrane domain. *Mol. Pharmacol.* 2003; 63:1051-1058.

Sheldon RS, Rahmberg M, Duff HJ. Quinidine/quinine: Stereospecific electrophysiologic and antiarrhythmic effects in a canine model of ventricular tachycardia. *J. of Cardiovas. Pharmacol.* 1990; 16:818-823.

#### **Pharmacokinetic**

Clohisy DR, Gibson TP. Comparison of pharmacokinetic parameters of intravenous quinidine and quinine in dogs. *J. Cardiovasc. Pharmacol.* 1982; 4:107-110.

Coleman MD, Timony G, Fleckenstein L. The disposition of quinine in the rat isolated perfused liver: Effect of dose size. *J. Pharm. Pharmacol.* 1990; 42:26-20.

Czuba MA, Morgan DJ, Ching MS, Mihaly GW, Ghabrial H, Hardy KJ, Smallwood RA. Disposition of the diastereoisomers quinine and quinidine in the ovine fetus. *J. Pharmaceut. Sci.* 1991; 80:445-448.

Mansor SM, Ward SA, Edwards G, Hoaksey PE, Breckenridge AM. The effect of malaria infection on the disposition of quinine and quinidine in the rat isolated perfused liver preparation. *J. Pharm. Pharmacol.* 1990; 42:428-432.

Mansor SM, Ward SA, Edwards G. The effect of fever on quinine and quinidine disposition in the rat. *J. Pharm. Pharmacol.* 1991; 43:705-708.

Mihaly GW, Hyman KM, Smallwood RA. High-performance liquid chromatographic analysis of quinine and its diastereoisomer quinidine. *J. Chromatog.* 1987; 415:177-182.

Onyeji CO, Dixon PAF, Ugwu NC. Disposition of quinine in rats with induced renal failure. *Pharmaceutisch Weekblad [Sci]*, 1992; 14:185-190.

Pussard E, Bernier A, Fouquet E, Bouree P. Quinine distribution in mice with *plasmodium berghei* malaria. *Eur. J. Drug Met. Pharmacokinetics.* 2003; 28:11-20.

Wanwimolruk S, Wong CWS, Ho PC. In-vitro hepatic metabolism of quinine in cow, pig and sheep. *Pharmaceut. Sci.* 1996; 2:295-298.

Zhang H, Ramsay N, Coville PF, Wanwimolruk S. Effect of erythromycin, rifampicin and isoniazid on the pharmacokinetics of quinine in rats. *Pharm. Pharmacol. Commun.* 1999; 5:467-472.

Zhang H, Wong CW, Coville PF, Wanwimolruk S. Effect of the grapefruit flavonoid naringin on pharmacokinetics of quinine in rats. *Drugs Met. Drug Interactions.* 2000; 17:351-363.

Zhao X-J, Ishizaki T. The *in vitro* hepatic metabolism of quinine in mice, rats and dogs: comparison with human liver microsomes. *J. Pharmacol. Exp. Ther.* 1997; 283:1168-1176.

### **Toxicology**

Colley JC, Edwards JA, Heywood R, Purser D. Toxicity studies with quinine hydrochloride. *Toxicol.* 1989; 54:219-226.

Flaks B. Effects of chronic oral dosing with quinine sulphate in the rat. *Path. Res. Pract.* 1978; 163:373-377.

King M-T, Beikirch H, Eckhardt K, Gocke E, Wild D. Mutagenicity studies with x-ray – contrast media, analgesics, antipyretics, antirheumatics and some other pharmaceutical drugs in bacterial, *Drosophila* and mammalian test systems. *Mutat. Res.* 1979; 66:33-43.

Lapointe G, Nosal G. Saccharin- or quinine-induced changes in the rat pups following prolonged ingestion by the dam. *Biol. Neonate.* 1979; 36:273-276.

Münzner R, Renner HW. Mutagenicity testing of quinine with submammalian and mammalian systems. *Toxicology.* 1983; 26:173-178.

Pussard E, Bernier A, Fouquet E, Bouree P. Quinine distribution in mice with *plasmodium berghei* malaria. *Eur. J. Drug Met. Pharmacokinetics.* 2003; 28:11-20.

Savini EC, Moulin MA, Herrou MDJ. Experimental study of the effects of quinine on rat, rabbit, and dog fetuses. *Therapie*, 1971; XXVI: 653-574. [FRENCH ORIGINAL; TRANSLATION]

Sideropoulos AS, Specht SM, Jones MT. Feasibility of testing DNA repair inhibitors for mutagenicity by a simple method. *Mut. Res.* 1980; 74:95-105.

Tanimura T. The use of non-human primates in research on human reproduction. WHO Research and Training Centre on Human Reproduction Karolinska Institutet (Symposium), Stockholm, 1972; 293-308.

**Studies not reviewed within this submission:**

**none**

## 2.6.2 PHARMACOLOGY

### 2.6.2.1 Brief summary

The mechanism of the antimalarial activity of quinine sulfate is not fully understood but is proposed to be *via* inhibition of the *P.falciparum* heme polymerase, required to convert heme to the non-toxic malaria pigment hemozoin. Quinine sulfate is highly concentrated in the *P.falciparum* acidic food vacuoles, the putative site of inhibition of the heme polymerase. It is unclear whether direct toxicity of the accumulated heme alone or involves a heme-quinine complex provides the antimalarial activity of quinine.

### 2.6.2.2 Primary pharmacodynamics

Mechanism of action: Antimicrobial activity against the blood schizont form of *P.falciparum*

Drug activity related to proposed indication: Quinine sulfate acts primarily on the blood schizont form of *P.falciparum*; it is not gametocidal and has little effect on the sporozoite or pre-erythrocytic forms. Because of this limited spectrum of antimalarial activity, quinine is not used for malaria prophylaxis. The antimalarial mechanism of action is proposed *via* inhibition of the *P.falciparum* heme polymerase, which is required to convert heme to the non-toxic malaria pigment hemozoin. Quinine sulfate is highly concentrated in the *P.falciparum* acidic food vacuoles, the putative site of inhibition of the heme polymerase. It is unclear whether direct toxicity of the accumulated heme alone or involves a heme-quinine complex provides the antimalarial activity of quinine.

### 2.6.2.3 Secondary pharmacodynamics

#### 2.6.2.4 Safety pharmacology

Effects on skeletal muscle, the auditory system, and glucose control are discussed following the cardiovascular subsection.

#### 5.2.1 Cardiovascular Effects

A stereoisomer of quinine, quinidine, was identified as the potent antiarrhythmic substances extracted from the plant. Quinidine has been used for that purpose since the early 1920's. Quinine shares many of its cardiac properties, however, quinine is less potent and of shorter duration. It was also recognized early that therapeutic doses of quinine have little if any effect on the normal heart or blood pressure, but that large doses result in vasodilatation-induced hypotension. The electrophysiology of quinidine's, and hence quinine's, effects on cardiac conduction have been well elucidated in *in vitro* and *in vivo* studies. At concentrations as low as 0.78 µg/mL, quinine is an open-state blocker of Na<sup>+</sup> channels (resulting in an increased threshold for excitability and decreased

automaticity) and blocks the rapid component of the K<sup>+</sup> channel delayed rectifier (*I<sub>kr</sub>*) (resulting in prolonged action potentials in most cardiac cells). As a result of these effects, refractoriness is prolonged in most tissues. At higher concentrations, quinine blocks the slow component of delayed rectifier, inward rectifier, transient outward current, and L-type Ca<sup>2+</sup> current. In the intact animal, quinine has been shown to produce  $\alpha$ -adrenergic receptor blockade and vagal inhibition.

Clinically, these properties may produce an increase in QRS duration and prolongation of the QT interval. Most of the agents that prolong QT intervals, prolong cardiac action potentials to a disproportionate extent when the underlying heart rate is slow. This effect, in turn, results in early after depolarizations (EADs) and related triggered activity *in vitro*, and can cause *torsades de pointes*. The effect of the drug on the PR interval is variable as the vagolytic effects tend to inhibit the direct depressant effect on AV nodal conduction. Moreover, quinine's vagolytic effect can result in increased AV nodal transmission of atrial tachycardias such as atrial flutter.

#### ***In vitro Studies***

The most recent published studies have focused on better understanding of the molecular basis for the observed *in vitro* effects on ion channels. Four studies that illustrate the comparative potencies and/or effects of quinine and quinidine are summarized below. In the paper by Michel and colleagues (Michel *et al.*, 2002), the authors pointed out that differences in tubular secretion and/or stereoselective interaction with organic cation transporters may account for the greater potency of quinidine rather than suggest differences in their actions on cardiac ion channels. References were cited that supported this suggestion. However, the results of the *in vitro* studies discussed below suggest a stereoselective interaction with cardiac receptors in cells or tissue of isolated systems. Quinine, like quinidine and chloroquine, causes voltage-dependent block of *I<sub>kr</sub>*. The poreforming  $\alpha$  subunits of channels that conduct the rapid delayed rectifier K<sup>+</sup> current are coded by the Human Ether-a-go-go-Related Gene (HERG). A series of studies were designed to elucidate the molecular mechanisms of HERG channel blockade (Sanchez-Chapula *et al.*, 2003). Blockade of wild type *Xenopus laevis* oocyte HERG by quinidine and quinine was enhanced by progressive membrane depolarization and accompanied by a negative shift in the voltage dependence of channel activation. Quinine was more than 10-fold less potent than quinidine: the median inhibitory concentration (IC<sub>50</sub>) values determined with voltage-clamp pulses to 0 mV were 3.6  $\mu$ g/mL and 44.6  $\mu$ g/mL for quinidine and quinine, respectively. By studying the effects of mutations of Y625A, Y625F, and V625A aromatic residues in the S6 domain of the HERG channel on voltage-dependent block, the study also showed the critical role for this putative binding site. Similar effects were seen for vesnarinone, an uncharged control drug that is not dependent on transmembrane voltage charge. These results suggest that voltage-dependent block of HERG is not a transmembrane field effect but rather gating-dependent changes in orientation of Y652.

Quinine and quinidine also have some other electrophysiological properties in common. Quinine and quinidine (each at 15.66  $\mu$ g/mL) have been shown to reduce, but not block, K<sup>+</sup> (*I<sub>to</sub>*) and Ca<sup>2+</sup> (*I<sub>ca</sub>*) currents in rat ventricular cardiomyocytes; only the latter effect was dependent on the frequency of stimulation, suggesting an effect on inactivated channels (Michel *et al.*, 2002). Peak current amplitude (*I<sub>max</sub>*) was reduced with EC<sub>50</sub> values of 8.61  $\mu$ g/mL and 11.75  $\mu$ g/mL, respectively, for the *I<sub>to</sub>* and 10.96  $\mu$ g/mL and 7.83  $\mu$ g/mL,

respectively, for  $I_{ca}$ . The authors also indicate and provide supporting references suggesting that the effects are on the outer surface of the cell membrane, interacting with open  $K^+$  channels and inactivated  $Ca^{2+}$ .

Because quinidine and quinine are enantiomers, their differences in activity are often attributed to stereoselective effects. Studies of both compounds in the rat aorta indicate that quinidine is three to five times more potent than quinine in inhibition of  $KCl$ - and norepinephrine -induced contractions (del Pozo *et al.*, 1996) and the difference was attributed to stereoselective effect in cardiac tissue. The stereoselective inhibition of contractions in the vascular smooth muscle was attributed to  $Ca^{2+}$  channel blockade and the competitive antagonism of norepinephrine -induced contractions were mediated by  $\alpha$ -adrenergic receptors. At high concentrations, the enantiomers also exerted a nonstereospecific inhibitory effect against contractions induced by 5-hydroxytryptamine (5-HT) and endothelin-1 (ET-1).

Utilizing whole-cell patch clamp techniques on rat isolated ventricular myocytes, (Clark *et al.*, 1995) the effects of quinidine sulfate (6  $\mu M$  or 5.5  $\mu g/mL$ ) were studied on rat ventricular resting action potentials, on the magnitude and rate of decay of the transient outward currents, on the "use-dependent" (channels are repetitively activated by a train of pulses) block of the transient outward current, and several studies designed to elucidate the kinetic basis of the use-dependent quinidine block. The results demonstrated that quinidine blocks two components of outward  $K^+$  current: a  $Ca^{2+}$ -insensitive transient current, and a slowly inactivating delayed rectifier-like component. Further, these effects of quinidine on  $I_{to}$  were shown to be augmented by membrane depolarization in rat cardiomyocytes. These findings (time and membrane potential dependence of this block) indicate a preferential interaction of quinidine with open  $K^+$  channels. Further, the work of Sanchez-Chapula and colleagues (Sanchez-Chapula *et al.*, 2003) demonstrated that the blocking effect of quinidine on  $K^+$  channels is voltage-dependent, increases with progressive membrane depolarization and serves to explain its propensity to induce long QT syndrome and *torsades de pointes* arrhythmia.

#### ***In vivo Studies***

Quinine, like quinidine, has been shown to possess antiarrhythmic activity in animal models. In a canine model of acute ischemia, both quinine and quinidine (each given as 10 mg/kg i.v.) were equally effective in reducing the incidence of ventricular fibrillation and tachycardia as well as the total number of premature beats (Jurkiewicz *et al.*, 1985). Only quinidine prolonged the QT interval.

Quinine and quinidine share several antiarrhythmic properties (Klevans *et al.*, 1977). In the models studied, the sole difference was acetylcholine (ACh)-induced atrial fibrillation, a model in which quinine was not active but quinidine was. The data show that both quinine and quinidine raised ventricular fibrillation thresholds in cats and decreased ouabain-induced premature ventricular complexes in dogs. In another canine model, however, quinine showed no antiarrhythmic activity and quinidine showed only weak antiarrhythmic activity.

A major electrophysiologic effect of quinidine and quinine is a prolongation of conduction time. Only quinidine prolongs action potential duration *in vitro* (Mirro *et al.*, 1981, as cited by Sheldon *et al.*, 1990) and repolarization time *in vivo* (Jurkiewicz *et al.*, 1985).

The electrophysiological events contributing to the antiarrhythmic effects of quinine and

quinidine have been studied in a canine model of inducible ventricular tachyarrhythmias late after occlusion-reperfusion (Sheldon *et al.*, 1990). The dosing regimen of quinidine was chosen to result in concentrations comparable to those that are effective in humans as an antiarrhythmic; a 29 mg/minute loading dose followed by a 1.4 mg/minute maintenance infusion resulted in concentrations of  $14 \pm 7 \mu\text{g/mL}$ . A quinine sulfate regimen was then chosen to result in similar plasma levels; a 16 mg/minute loading dose followed by a maintenance infusion of 2.2 mg/minute resulted in plasma concentrations of  $18 \pm 6 \mu\text{g/mL}$ . There was a mean increase in conduction time of 14 msec with both drugs. Quinidine prolonged local repolarization times and refractoriness much more than quinine. Sustained ventricular tachyarrhythmia was consistently inducible during saline studies. Antiarrhythmic activity was observed in 3 out of 12 quinidine-treated dogs, but in none of 15 quinine- or 13 saline-treated dogs. Quinidine also significantly prolonged monomorphic ventricular tachycardia cycle length while quinine had no significant effect.

The results indicate that prolonging refractoriness is important in preventing the induction of ventricular tachyarrhythmia and in prolonging ventricular tachycardia cycle length. Quinine did not show antiarrhythmic activity in this canine model at mean plasma concentration of  $18 \mu\text{g/mL}$  (a concentration that exceeds those achieved clinically in humans in treating malaria).

In an old report (Hiatt *et al.*, 1948), hemodynamic effects of oral quinine were studied in normal and neurogenic-induced-hypertensive dogs. Following three or four times daily dosing of quinine sulfate or quinidine (10 to 15 mg/kg) for several days, there were no significant effects on the blood pressure in normal dogs, but there were hypotensive effects in neurogenic-induced-hypertensive dogs, with a more marked response to quinidine. Heart rate was increased in normal dogs, but the neurogenic-induced hypertensive dogs, which already had a rapid heart rate, showed either further acceleration or no change. Renal blood flow and glomerular filtration rate (GFR) increased in both normal and hypertensive dogs, but quinine caused a greater renal hyperemia suggesting a vasodilating effect. The effects discussed above were observed with quinine plasma concentrations from 1 to 4  $\mu\text{g/mL}$ .

One study (Mecca *et al.*, 1980) demonstrated that i.v. quinine reduces the mean arterial blood pressure, increases pulse pressure and inhibits the pressor effects of norepinephrine. They further evaluated the  $\alpha$ -adrenergic blocking properties of quinine in the anesthetized dog. Intravenous infusion of quinine was shown to decrease blood pressure and increase heart rate and myocardial contractility, and to reduce pressor responses to adrenergic agents. In 20 anesthetized mongrel dogs, quinine HCl (50 mg/kg, i.v.) infused over a 20-minute period produced a 22% maximum decrease in diastolic blood pressure, a 5% increase in systolic pressure, and a 52% increase in myocardial contractile force; pulse pressure was increased 53% by the end of the infusion. The initial positive inotropic response was maximal in the first 5 to 15 minutes of the quinine infusion and decreased to near control levels 40 minutes following the quinine infusion. The effects of quinine on the cardiovascular responses to a variety of cardioactive and vasoactive agents were also studied. Quinine caused a marked reduction in norepinephrine pressor response, blockade of the epinephrine pressor response, partial blunting of the angiotensin II pressor effect but no change in the depressor effect of isoprenaline. This is consistent with observations in separate *in vitro* studies performed

using rabbit aorta strips. Quinine 15.7, 54.8, or 78.3  $\mu\text{g/mL}$  caused a parallel dose-related shift of the noradrenaline dose-response curve to the right; maximum contractile response was not altered. Quinine had no effect on responses to histamine or angiotensin II. The alterations in myocardial contractile process may involve inhibition of intracellular  $\text{Ca}^{2+}$  reuptake.

Neurological effects:

Not included in material provided

Cardiovascular effects:

Studies in the early 19<sup>th</sup> century identified quinidine, a stereoisomer of quinine, as the most potent of the antiarrhythmic substances extracted from the plant. Quinidine has been used for that purpose since the early 1920's. Quinine shares many, if not all of its cardiac properties, however, quinine is less potent and of shorter duration. It was also recognized early that therapeutic doses of quinine have little if any effect on the normal heart or blood pressure, but that large doses result in vasodilatation-induced hypotension. The electrophysiology of quinidine's, and hence quinine's, effects on cardiac conduction have been well elucidated in *in vitro* and *in vivo* studies. At concentrations as low as 0.78  $\mu\text{g/mL}$ , quinine is an open-state blocker of  $\text{Na}^+$  channels (resulting in an increased threshold for excitability and decreased automaticity) and blocks the rapid component of the  $\text{K}^+$  channel delayed rectifier ( $I_{kr}$ ) (resulting in prolonged action potentials in most cardiac cells). As a result of these effects, refractoriness is prolonged in most tissues. At higher concentrations, quinine blocks the slow component of delayed rectifier, inward rectifier, transient outward current, and L-type  $\text{Ca}^{2+}$  current. In the intact animal, quinine has been shown to produce  $\alpha$ -adrenergic receptor blockade and vagal inhibition. Clinically, these properties can result in a modest increase in QRS duration and prolongation of the QT interval. Most of the agents that prolong QT intervals, prolong cardiac action potentials to a disproportionate extent when the underlying heart rate is slow. Subsequently, this produces polarizations (EADs) and related triggered activity *in vitro*, and can cause *torsades de pointes*. The effect of the drug on the PR interval is variable as the vagolytic effects tend to inhibit the direct depressant effect on AV nodal conduction. The vagolytic effect of quinine can result in increased AV nodal transmission of atrial tachycardias such as atrial flutter.

Pulmonary effects: No studies or literature were provided regarding pulmonary effects of quinine.

Renal effects: No studies or literature were provided regarding renal effects of quinine.

Gastrointestinal effects: No studies or literature were provided regarding gastrointestinal effects of quinine

Abuse liability: No studies or literature were provided regarding the abuse liability of quinine  
There is no known risk for abuse.

Other:

**Hypoglycemic Effects**

Hypoglycemia and hyperinsulinemia occur in malaria patients treated with quinine. *In vitro*, quinine potentiated glucose-induced insulin release by isolated rat islets (Henguin *et al.*, 1975, as cited by Okitolonda *et al.*, 1986).

In normal rats, a hypoglycemic effect was not observed following single or chronic administration of quinine; however, insulin was increased (Okitolonda *et al.*, 1986). Single intraperitoneal injection of quinine (30 mg/kg) transiently increased plasma glucose and insulin levels. In another chronic study, male albino rats received a daily dose of 10 or 30 mg/kg of quinine in the drinking water for 20 weeks. Basal plasma insulin levels were increased in treated rats, while plasma glucose levels were only slightly and not consistently decreased. The insulin content of the pancreas was not affected by quinine treatment. After oral or intravenous administration of glucose, the plasma insulin levels were higher and the disappearance rate of glucose was greater in rats receiving quinine than in the control. The study showed that chronic oral administration of quinine did not cause hypoglycemia in normal rats, but caused hyperinsulinemia and accelerated disposal of oral or intravenous glucose.

**Skeletal Muscle**

Quinine is known to increase the refractory period of skeletal muscle by direct action on the muscle fiber and the distribution of calcium within the muscle fiber, thereby diminishing the response to tetanic stimulation. It also decreases the excitability of the motor end-plate region, reducing the responses to repetitive nerve stimulation and to acetylcholine. Quinine can antagonize the action of physostigmine on skeletal muscle as effectively as curare. These are the bases for the purported utility in the off-label treatment of nocturnal leg cramps.

2.6.2.5 . **Pharmacodynamic drug interactions** Preclinical drug interaction studies were not referenced

**2.6.3 PHARMACOLOGY TABULATED SUMMARY**

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Table 5.1  
*In vitro* Effects of Quinine on Cardiac Ion Channels

Reference	Species/ Strain	N / Sex / Group	Parameters	Results / Observations
<b>Cardiovascular Effects</b>				
<u>Clark et al., 1995</u>	Ventricular Cardiomyocytes from the rat (Strain unspecified)	<i>in vitro</i> (6-8 replicates)	patch-clamp technique	Quinidine-induced blockade of transient outward K <sup>+</sup> current as evidenced by: <ul style="list-style-type: none"> <li>• increased rate of decay of current,</li> <li>• use-dependent inhibition and slowing of recovery from inactivation in the presence of quinidine.</li> </ul> In the case of Ca <sup>2+</sup> -independent transient outward current, the mechanism is an open channel block by ionized quinidine.
<u>del Pozo et al., 1996</u>	Isolated thoracic aortic rings from male Sprague-Dawley rat	<i>in vitro</i> (6 replicates)	Contractile responses induced by KCl, NEPI, 5-HT and ET-1	Quinine was 4-5-fold less potent than quinidine when used to relax KCl-induced contractions. The inhibitory effects of quinidine were voltage-dependent and those of quinine were not. At low concentrations, quinine was 3-4 times less potent than quinidine in inhibiting contractions induced by NEPI and at the highest concentrations, quinine (97 µg/mL as base) was comparable to quinidine (65 µg/mL). The inhibitory effects of quinidine and quinine on contractions induced by 5-HT and ET-1 were not stereoselective. Both enantiomers inhibited contractions elicited by 5-HT in Ca <sup>2+</sup> -free media, attributed to Ca <sup>2+</sup> channel blocking properties.
<u>Michel et al., 2002</u>	Cardiomyocytes from Sprague-Dawley rat	<i>in vitro</i> (6-9 replicates)	patch-clamp technique	Quinine and quinidine depressed transient outward current (IC <sub>50</sub> : 8.16 & 11.75 µg/mL, resp.) and L-type Ca <sup>2+</sup> current (IC <sub>50</sub> : 11 & 7.8 µg/mL, resp.); exerted their effects on the outer surface of the cell membrane, interacted with open K <sup>+</sup> channels and inactivated Ca <sup>2+</sup> channels.
<u>Sanchez-Chapula et al., 2003</u>	Xenopus laevis oocyte with mutant channels	<i>in vitro</i> (5 replicates)	HERG delayed rectifier K <sup>+</sup> channel blocking activity	Quinine, quinidine and chloroquine blocked HERG channels and blockade enhanced by membrane depolarization. Quinine was 12-fold less potent than quinidine.  IC <sub>50</sub> values: (with voltage-clamp pulses to 0 mV) were: Quinine: 44.6 µg/mL (57 µM) Quinidine: 3.6 µg/mL (4.6 µM)

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Table 5.2  
*In vivo* Antiarrhythmic Activity of Quinine and Quinidine (Klevans et al., 1977)

Species (N)	Model	Dose	Results
Dog (N = 11)	Duration of Ach-induced atrial fibrillation	2, 4, 6, 8, 20 mg/kg. 3-minute i.v. infusion	Quinine had no effect Significant ↓ with quinidine ≥ 6 mg/kg
Dog (N = 5/group)	Reversal of aconitine-induced atrial fibrillation	2, 4, 8 mg/kg. 3-minute i.v. infusion every 10 minutes	Similar dose-dependent reductions in atrial rate for both drugs
	Ouabain-induced abnormal ventricular beats (3 hours after aconitine experiment)	2, 4, 8 mg/kg. 3-minute i.v. infusion every 10 minutes	↓ abnormal beats for both drugs
Dog (N = 5/group)	Electrical stimulus-induced premature atrial depolarization and His-Purkinje conduction time	10 mg/kg i.v.	↑ atrial refractoriness and conduction time, more long-lasting with quinidine
Cat (N = 5/group)	Electrical stimulus-induced ventricular fibrillation threshold (VFT)	2, 6 mg/kg i.v.	↑ VFT after 6 mg/kg for both drugs (as compared to control); more long-lasting with quinidine

Table 5.4  
*In vivo* Cardiovascular Safety Pharmacology Studies of Quinine

Reference	Species/ Strain	N / Sex /Group	Dose / Duration / Route	Parameters	Results / Observations
<u>Mecca et al.</u> 1980	Dog	5	2.5 mg/kg/min infusion over 20 min (50 mg/kg total)	BP, pulse pressure, contractile force; dose-response obtained before and 30 min and 90 min after infusion	<p><u>Direct CV effects:</u>            Diastolic BP: 22% decrease            Pulse pressure: 53% increase            Myocardial contractile force: 52% increase.</p> <p><u>Modulation of other agents:</u>            Norepinephrine-, epinephrine- and angiotensin II- pressor responses were reduced or blunted. Isoprenaline-induced depressor response was not affected.</p> <p>CaCl<sub>2</sub>-positive inotropic effect was reduced.</p> <p>These effects are consistent with alpha-adrenergic blocking activity and modified catecholamine- and calcium-induced contractility.</p>
<u>Sheldon et al.</u> 1980	Mongrel dog (ischemic model)	24 total	16 mg/min x 18 min, then 2.2 mg/min (targeted to result in same extent of conduction prolongation as quinidine)	inducible ventricular tachyarrhythmia	<p><u>Antiarrhythmic activity</u> observed in 3/12 quinidine dogs; but no quinine or saline treated dogs</p> <p>Quinine 18 µg/mL and quinidine 14 µg/mL prolonged conduction times to similar extent, but quinidine prolonged local repolarization time; and refractoriness much more than quinine.</p> <p>Monomorphic ventricular tachycardia cycle length: significantly prolonged by quinidine, but not quinine.</p>

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## 2.6.4 PHARMACOKINETICS/TOXICOKINETICS

### 2.6.4.1 Brief summary

Nonclinical pharmacokinetics of quinine are generally similar among animal species and humans. Quinine is absorbed following oral administration in animals. Oral bioavailability in the rat has been estimated to be approximately 17%. The drug binds to plasma protein to a moderate extent (approximately 60 to 80%). In humans, binding is mainly to  $\alpha_1$ -acidic glycoprotein. Quinine has been shown to cross the placental barrier in sheep with fetal levels less than 10% of maternal levels. In mice, rats, dogs, pigs, sheep as well as in humans, quinine is extensively metabolized in the liver, producing a major metabolite, 3-hydroxyquinine. CYP<sub>450</sub> 3A isozyme was identified as the main enzyme for the production of 3-hydroxyquinine. In rats, systemic exposure to 3-hydroxyquinine was three-fold greater than to that of quinine. In rats, naringin (grapefruit flavonoid and inhibitor of CYP3A4) was shown to increase plasma concentration and systemic exposure to orally administered quinine, but another inhibitor, erythromycin, had no effect. In rats, a total of 50% of the administered dose was excreted in urine in 24 hours: renal excretions of quinine and 3-hydroxyquinine accounted for approximately 16% and 34% of the administered dose, respectively. In rodent malaria models, the clearance and systemic distribution are decreased and both plasma concentration and systemic exposure are increased.

### 2.6.4.2 Methods of Analysis

[see under individual study reviews]

### 2.6.4.3 Absorption

In a pilot dose-selection study, mice were injected with quinine in doses that ranged from 20 to 200 mg/kg i.p. Whole blood levels were measured 15 minutes post-dose. Concentrations increased with dose in a more than proportional fashion as illustrated in the table below.

### 2.6.4.4 Distribution

The *in vitro* binding of quinine was studied in rat plasma at concentrations of 1 and 5  $\mu\text{g/mL}$  (Mansor *et al.*, 1991). Binding was 21.0 to 21.8%, regardless of temperature (19 to 44°C).

Quinine concentrates in erythrocytes, as was evident in Table 5.9 (Pussard *et al.*, 2003). RBC levels in mice are approximately twice as high as whole blood concentrations. Plasma concentrations are lower, of which approximately 60% is protein bound. The same animals were sacrificed 2 hours after the 80-mg/kg i.v. dose, selected tissues harvested and homogenized, and supernatant removed (Pussard *et al.*, 2003). The highest levels of quinine were present in the spleen, lungs, and kidney, with tissue-to-whole blood levels similar in infected and non-infected animals; tissue-to-free fraction levels were higher in the high-infection group. Drug was also distributed to the liver, heart, and muscle, with levels higher in infected animals only in the latter two tissues. Distribution

to the brain was low and did not change with infection.

Quinine has been shown to cross the placental barrier in two studies performed in pregnant sheep. Following a single 5-minute intravenous infusion of quinine HCl (10 mg/kg) to a pregnant sheep (Mihaly *et al.*, 1987), fetal exposure was 6 to 7% of the maternal exposure. The AUC<sub>0-inf</sub> values for quinine were 9091 (mother) and 587 (fetus) µg·hr/mL. The half-life was approximately 1.1 and 1.3 hours for the ewe and fetus, respectively.

In another study (Czuba *et al.*, 1991), fetal exposure was measured at steady state (following a 5.12 mg/kg bolus and 2.71 mg/kg/hour intravenous infusion). Fetal total concentrations of quinine were 8 to 10% of those in the mother.

Similarly, fetal unbound concentrations were substantially lower than maternal unbound concentrations. Plasma protein binding of quinine was approximately 85% in the mother and 44% in the fetus. Maternal systemic clearance of quinine was higher than in the fetus, and in both, renal clearance represented less than 2% of total clearance.

#### 2.6.4.5 Metabolism

Quinine is extensively metabolized in the liver in animal species and humans. Hepatic CYP<sub>450</sub> 3A isozyme catalyzing 3-hydroxylation has been shown as the main enzyme in both laboratory and domestic animal species. Quinine is oxidized to 3-hydroxyquinine, a major metabolite, in mice, rats, dogs, pig, sheep, and humans. In humans, CYP3A4 has been identified as the main enzyme in the metabolism of quinine, with CYP2C19 also contributing to some extent. In addition to 3-hydroxyquinine, additional polar metabolites were detected in the plasma. The relevant data are summarized below.

##### *In Vitro Studies*

Quinine metabolism was studied (Zhao *et al.*, 1997) using microsomes from male mouse (Crj/CD), rat (Wistar), dog (Beagle), and human livers. Human liver microsomes were prepared from non-tumor bearing sections of human liver obtained during partial hepatectomy. Following incubation of microsomal fractions with an NADPH-generating system and 0.39 to 470 µg/mL quinine at 37°C for 10 to 15 minutes, the supernatant was prepared and 3-hydroxyquinine was determined by HPLC.

3-Hydroxyquinine was the major metabolite peak observed. It was formed in a monophasic fashion in all species but the mouse, in which formation was biphasic. In a series of studies, co-incubation with the inhibitors ketoconazole and troleandomycin, demonstrated that CYP3A is involved as *p*-nitrophenol, *s*-mephenytoin, and sulfaphenazole all inhibited formation by more than 50%. For the same reason (inhibition by *p*-nitrophenol), CYP2C19 may also have a minor role in human metabolism. This role of CYP3A was confirmed by the use of specific antibodies and the determination of a correlation between CYP3A and content and hydroxylation in human microsomes.

Chloroquine inhibited quinine 3-hydroxylation by rat and dog liver microsomes but not by human liver microsomes. Primaquine, doxycycline, and tetracycline inhibited formation in all three species.

Similar results were obtained in an earlier *in vitro* metabolism study (Wanwimolruk *et al.*, 1996) in domestic animals. Quinine HCl was incubated with liver microsomes from cow, pig and sheep. 3-hydroxyquinine was shown to be the major metabolite and CYP3A was the major enzyme involved in the formation of this metabolite, because

troleandomycin and midazolam (known CYP3A inhibitors) inhibited this biotransformation in these species.

#### ***Ex Vivo Study***

At least two studies of isolated perfused rat livers have been published, one of which investigated the effects of malaria infections. The latter study was reviewed earlier. Dose-dependent kinetics were observed in rat (male Sprague-Dawley) isolated perfused liver (Coleman *et al.*, 1990). Hepatic extraction was studied by taking simultaneous samples from the portal inflow and the inferior vena cava outflow (5 livers per dose group). Samples of perfusate and bile and liver homogenates were analyzed for quinine concentrations by HPLC. As quinine concentrations increased (6.25 to 25 mg added to 100 mL perfusate, infusing at a rate of 15 mL/minute), there were dose-related decreases of hepatic extraction and clearance and an increase of terminal half-life. Plasma concentrations of total and free quinine decayed biexponentially over 4 hours. Exposure and half-life increased with dose in a greater than proportional fashion. Ten minutes post-dose, extraction of quinine at 6.25 mg was 56.5%, twice that at the highest dose (25.0%). Elimination of unchanged quinine in the bile was low, with less than 1% of the parent drug being detected. Relatively little parent drug was recovered from the liver at 4 hours, at 25 mg, less than 6%. Polar metabolites of quinine were detected in the bile and liver homogenates.

#### ***In Vivo Studies***

The effects of known CYP3A4 inhibitors (grapefruit flavonoid, naringin, and erythromycin) and inducers (rifampicin and isoniazid) have been studied with varying results. Female Wistar rats were pretreated with naringin 25 mg/kg once a day for 7 consecutive days; on study Day 1, quinine HCl (25 mg/kg) was administered either by the oral or i.v. route (Zhang *et al.*, 2000). Control groups of non-pretreated animals were similarly dosed. Blood samples were collected for up to 6 hours after quinine administration and plasma quinine concentrations assayed by a HPLC. After oral quinine administration, pretreatment with naringin led to a 208% increase in  $C_{max}$ , 93% increase in  $T_{max}$  and a 152% increase in the AUC. There was no effect following i.v. dosing. The oral bioavailability of quinine was significantly increased from 17% in the control group to 42% in naringin-pretreated group.

The effects of erythromycin, an inhibitor of CYP3A4, and rifampicin and isoniazid, inducers of CYP3A4 on the pharmacokinetics of quinine (25 mg/kg i.v.) were also studied (Zhang *et al.*, 1999). Male Wistar rats were divided into groups and pretreated with erythromycin (80 mg/kg p.o.) for 7 days. Control rats received vehicle (corn oil) pretreatment. There were no significant differences in the pharmacokinetics of quinine between the two groups. Similarly, all Wistar rats were pre-treated with rifampicin (30 mg/kg, p.o.), isoniazid (25 mg/kg, p.o.) and a combination of rifampicin and isoniazid (or corn oil), once a day for 14 days before receiving an i.v. bolus dose of quinine bisulfate (25 mg/kg). Blood samples were collected up to 6 hours after quinine administration and assayed by HPLC. There were no significant differences in the pharmacokinetic parameters of quinine between pretreated and control groups.

#### **2.6.4.6 Excretion**

Quinine is excreted in the urine as unchanged parent drug and the 3-hydroxyquinine metabolite. Following administration of quinine sulfate (10 mg/kg, i.p.), approximately

50% of the dose is excreted in rat urine in 24 hours: approximately 16% and 34% of the dose was excreted as quinine and 3-hydroxyquinine, respectively (Onyeji *et al.*, 1992). Another metabolite was present but not identified, due to a lack of suitable HPLC reference compound.

When acute renal failure is induced, renal clearance (and hence total clearance) is decreased: systemic exposure to both the parent drug and the major metabolite (3-hydroxyquinine) is significantly increased and percents of the dose excreted in the urine as parent drug and metabolites are decreased. Results for three rat models of chemically induced renal failure are presented in the table below. Glycerol results in renal ischemia, gentamicin causes nephrotoxicity of the proximal convoluted tubules, and mercuric chloride produces widespread necrosis.

Renal clearance accounts for less than 20% of the total clearance. Other factors are likely to contribute to the increased exposure. The volume of distribution is decreased, postulated to be due to an increased plasma protein binding (as has been seen for man and, with quinidine, for rats) and therefore lower free fraction. The reduced plasma levels of 3-hydroxyquinine are also consistent with a lower free fraction.

#### 2.6.4.7 Pharmacokinetic drug interactions

not conducted or referenced

#### 2.6.4.8 Other Pharmacokinetic Studies

No t conducted or referenced

#### 2.6.4.9 Discussion and Conclusions

Quinine plasma levels are best fit by two- or three-compartment pharmacokinetic models. In one study (Mansor *et al.*, 1991), separate group of rats received quinidine in the same dose; pharmacokinetic parameter values were similar. The disposition of quinine in non-infected and *P. berghei*-infected mice has been compared (Pussard *et al.*, 2003). A dose of 80 mg/kg i.p. was chosen as the one giving concentrations close to those that are clinically active. Clearance is lower in infected animals, resulting in a higher systemic exposure. Whole blood and erythrocyte concentrations as well as AUC<sub>0-inf</sub> of quinine were higher with a higher level of parasitemia; hematocrit, however, was lower. Based on the results of an *in vitro* perfused liver study (Mansor *et al.*, 1990), the altered clearance in infected animals is not solely attributable to differences in red blood cell (RBC) distribution. Livers isolated from young rats infected with merozoites of *P. berghei*, a rodent malaria model, and from non-infected control rats were perfused for 4 hours with a solution of quinine 1 mg in 100 mL. Quinine exposure was statistically significantly higher in livers from infected animals (AUCs of 6470 ± 1101 versus 3822 ± 347 ng-hr/mL for infected and non-infected, respectively) and quinine clearance was decreased (0.33 ± 0.08 versus 0.64 ± 0.09 mL/min/gm liver, respectively). Fever, which results in increased cardiac output and hepatic blood flow, has been postulated as a possible factor in the altered pharmacokinetic disposition of quinine. When fever was experimentally induced in rats by injection of *E. coli* lipopolysaccharide, clearance was significantly increased (226%), as was volume of distribution (47%) as compared with controls (Mansor *et al.*, 1991). Correspondingly, half-life was decreased

from 15.9 hours to 7.1 hours. Temperature did not alter protein binding *in vitro*. Pharmacokinetic investigations have been reported in several other species as well. Following a single i.v. dose (10 mg/kg over 5 minutes), in a pregnant ewe, plasma clearance was 9.4 mL/minutes; half-life was 1.1 hour (Mihaly *et al.*, 1987). In pregnant sheep following a bolus (8.85 mg/kg) followed by a constant intravenous infusion (5.59 mg/kg/hour), the mean maternal systemic clearance at steady state was 714 mL/minute and mean renal clearance was 6.58 mL/minute. Plasma protein binding was 84% (Czuba *et al.*, 1991). In both studies, quinine clearance tended to be higher than quinidine's.

In summary, quinine is absorbed following oral administration, concentrating in erythrocytes and distributing to the major organs of liver, heart, lung kidneys and muscle; distribution is increased in infected animals. Brain distribution is low and unaffected by infection. Placental transfer of quinine occurs; in sheep, the fetal exposure level is less than 10% of the maternal exposure. The drug is rapidly excreted from the body, with plasma half-lives in mice, rats and dogs of approximately 1, 13 to 16, and 7 hours, respectively. In mice, clearance is decreased and exposure is increased in infected animals. Quinine undergoes extensive metabolism in the liver in all animal species tested. The major metabolite is 3-hydroxyquinine; there are other unidentified metabolites. *In vitro* studies showed CYP450 3A isozyme is the responsible enzyme in animal species and humans. Unchanged drug accounts for approximately 10 to 20% of the administered dose in urine in the first 24 hours and half-life is prolonged in models of acute renal failure. Urine and feces are routes of excretion.

#### 2.6.4.10 Tables and figures to include comparative TK summary

Pharmacokinetic Parameters of Quinine Determined in Non-Infected Animals after a Single Intravenous (i.v.), Intraperitoneal (i.p.) or Oral (p.o.) Doses

Species/Strain	Mouse <sup>1</sup> Swiss OF-1	Rat <sup>2</sup> Wistar	Rat <sup>3</sup> Wistar	Dog <sup>4</sup> Mongrel (N=7)
Quinine salt	Quinine HCl	Quinine sulfate	Quinine diHCl	Quinine HCl
Dose (mg base/kg)	80 (N=8)	10 (N=300)	50 (N=6)	6.5
Route of administration	i.p.	i.p.	p.o.	i.v. infusion (45 minutes)
C <sub>max</sub> (µg/mL)	8.6	NA	NA	NA
AUC <sub>0-∞</sub> (µg-hr/mL)	16.6	47	NA	NA
T <sub>1/2</sub> (h)	1.0	12.6	15.9	6.6
CL/F (mL/min/kg)	76.5	Plasma: 3.55 Renal: 0.62	23.0	8.11
Vd (L/kg)	NA	3.87	28.9	3.74

1 Source: Pussard *et al.*, 2003

2 Source: Onvani *et al.*, 1992

3 Source: Mansori *et al.*, 1991

4 Source: Clohiser *et al.*, 1982

NA: not available

\*based on average 30 g weight: reported as 153 L/hr

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## 2.6.5 PHARMACOKINETICS TABULATED SUMMARY

Pharmacokinetic Parameter Values of Quinine after Intravenous (i.v.) and Oral Administration of Quinine in the Rat with and without Naringin<sup>1</sup>

Parameter (unit)	i.v. Quinine		Oral Quinine	
	Control (N = 8)	Naringin (N = 9)	Control (N = 6)	Naringin (N = 7)
C <sub>max</sub> (µg/mL)	--	--	0.2 ± 0.1	0.7 ± 0.4*
T <sub>max</sub> (min)	--	--	35.0 ± 12.2	57.8 ± 30.5*
AUC (mg·h/L)	4.5 ± 1.0	4.5 ± 1.8	0.8 ± 0.4	1.9 ± 0.6*
F (%)	--	--	17.1	42.0
CL (L/h/kg)	5.9 ± 1.5	6.6 ± 3.0	--	--
CL/F (L/h/kg)	--	--	44.0 ± 25.9	14.1 ± 4.6*
V <sub>d</sub> (L/kg)	10.1 ± 4.0	11.1 ± 10.9	--	--
T <sub>1/2β</sub> (h)	1.2 ± 0.4	1.2 ± 0.9	1.3 ± 1.1	1.3 ± 0.7

Source: Zhang *et al.*, 2000.

Values are mean ± S.D. \*P &lt; 0.05.

<sup>1</sup>A grapefruit flavonoid that is a known inhibitor of CYP3A4Comparative Effects of Three Chemical Inducers of Renal Failure on the Pharmacokinetics of Quinine in the Rat<sup>1,2</sup>

Models of Induced Renal Failure				
	Control group Vehicle	Glycerol	Gentamicin	Mercuric chloride
Quinine				
AUC <sub>0-12h</sub> (µg·h/mL)	47	258	332	211
C <sub>max</sub> (µg/mL)	8.4 ± 2.3	17.8 ± 3.9	20.3 ± 4.3	15.1 ± 4.1
T <sub>max</sub> (h)*	3	3	3	3
CL/F <sub>r</sub> (mL/min·kg)	3.55	0.65	0.50	0.79
CL/F <sub>e</sub> (mL/min·kg)	0.62	0.052	0.041	0.080
V/F (L/kg)	3.87	1.86	1.76	1.90
T <sub>1/2</sub> (h)	12.6	32.6	41.2	27.5
% Dose in 24 h urine	15.62 ± 3.81	5.96 ± 2.41	5.90 ± 1.80	7.80 ± 2.51
3-Hydroxyquinine				
AUC <sub>0-12h</sub> (µg·h/mL)	138	81	90	72
% Dose in 24 h urine	33.80 ± 4.10	2.04 ± 1.22	1.62 ± 0.91	2.90 ± 1.13

<sup>1</sup> Source: Onyiah *et al.*, 1992.<sup>2</sup> Quinine sulfate was administered intraperitoneally as a single-dose of 10 mg/kg (N = 6/group)Comparative Effects of Three Chemical Inducers of Renal Failure on the Pharmacokinetics of Quinine in the Rat<sup>1,2</sup>

Models of Induced Renal Failure				
	Control group Vehicle	Glycerol	Gentamicin	Mercuric chloride
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<sup>1</sup> Source: Onyiah *et al.*, 1992.<sup>2</sup> Quinine sulfate was administered intraperitoneally as a single-dose of 10 mg/kg (N = 6/group)

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**Pharmacokinetic Parameter Values of *in vitro* Quinine 3-Hydroxylation in Microsomes from Several Species (Zhao *et al.*, 1997)**

Source of Microsomes	N	$K_m$ ( $\mu\text{g/mL}$ )	$V_{max}$ ( $\text{nmol/mg/mL}$ )	$V_{max}/K_m$ ( $\mu\text{L/mg/min}$ )
Mouse	7	23.3 + 4.4	2.39 ± 0.67	82.1 + 22.8
Rat	6	17.8 + 3.	1.94 + 0.25	86.3 + 11.2
Dog	5	108 + 17	0.77 + 0.26	5.6 + 1.8
Human	12	82 + 14	1.31 + 0.44	12.7 + 4.7

**Dose Proportionality of Quinine i.p. in Uninfected Mice (Pussard *et al.*, 2003)**

Dose (mg Base/kg)	N	Whole Blood $C_{15\text{min}}$ ( $\mu\text{g/mL}$ )
20	6	2.1 ± 0.7
50	6	4.4 ± 0.8
80	6	8.3 ± 2.5
120	6	14.8 ± 3.8
160	3 (3 died)	19.0 ± 3.8
200	3 (2 died)	31.6 ± 3.9

**Pharmacokinetic Parameter Values of Whole Blood Quinine in Mice with and without *P. berghei* Malaria<sup>1</sup>**

	Control group Non-infected (N=8)	Group 1 Infected-low (N=8)	Group 2 Infected-high (N=8)	P-value
Parasitemia (%) <sup>a</sup>	0	6.7 (5.0-20.0) <sup>a</sup>	30.0 (25.0-45.0) <sup>a,b</sup>	
AUC <sub>0-24</sub> ( $\mu\text{g}\cdot\text{h/mL}$ )	16.6 ± 5.1	35.6 ± 13.6 <sup>a</sup>	53.7 ± 14.1 <sup>a,b</sup>	0.002
$C_{max}$ ( $\mu\text{g/mL}$ )	8.6 (5.0-10.7)	9.1 (6.0-15.0)	15.0 (10.0-20.5) <sup>a,b</sup>	<0.001
$T_{max}$ (h) <sup>a</sup>	0.25 (0.25-0.50)	0.50 (0.25-0.50)	0.38 (0.25-0.50)	NS
CL/F (L/h)	153.0 ± 31.3	73.7 ± 32.1 <sup>a</sup>	47.5 ± 18.4 <sup>a,b</sup>	0.006
V/F (L)	213.0 ± 53.1	230.2 ± 65.4	162.8 ± 45.7 <sup>a</sup>	0.02
$T_{1/2}$ (h)	1.0 ± 0.3	3.6 ± 1.0 <sup>a</sup>	3.2 ± 0.6 <sup>a</sup>	NS

<sup>1</sup> Source: Pussard *et al.*, 2003<sup>a</sup> Expressed as median and range

<sup>a</sup> p<0.05 vs control group

<sup>b</sup> p<0.05 vs low-infected group

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Mean Quinine Concentrations in Blood Fractions 2 Hours Post-injection in Control and *P. berghei*-infected Mice<sup>1</sup>

	Control group Non-infected (N=8)	Group 1 Infected-low (N=25)	Group 2 Infected-high (N=22)	P-value
Parasitemia (%) <sup>a</sup>	0	11 (3-20)	30 (25-50)	
Hematocrit (%)	49.1 ± 2.6	41.6 ± 5.4 *	35.4 ± 6.0 **	<<0.001
Whole blood (µg/mL)	3.2 ± 0.7	6.4 ± 1.5 *	9.1 ± 2.4 **	<<0.001
Erythrocytes (µg/mL)	5.3 ± 1.3	11.3 ± 4.8 *	19.6 ± 7.9 **	<<0.001
Plasma total (µg/mL)	2.1 ± 0.5	3.8 ± 0.9 *	4.3 ± 1.0 *	0.05
Plasma free (µg/mL)	0.8 ± 0.2	1.2 ± 0.3 *	1.2 ± 0.3 *	0.04
Plasma free fraction (%)	38 ± 5	31 ± 6 *	28 ± 4 *	NS

<sup>1</sup> Source: Pizzard *et al.*, 2003

<sup>a</sup> Expressed as median and range

\* p<0.05 vs control group

\*\* p<0.05 vs low-infected group

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## 2.6.6 TOXICOLOGY

### 2.6.6.1 Overall toxicology summary

#### General toxicology:

Repeated dose toxicity studies have been performed in rats. The chronic toxicity of quinine sulfate include high rate of mortality and adverse liver effects. The duration-related liver toxicities include periportal glycogen depletion in all lobular areas and mild periportal fibrosis, small areas of cholangiofibrosis, Kupffer cells with varying degrees of lipid accumulation in the form of large cytoplasmic droplets, abundant binucleate hepatocytes, and increased numbers of lysosomes. There was no hyperplasia or evidence of liver necrosis in the treated animals. No liver tumors were reported. The study was conducted in male Leeds rats with quinine sulfate in drinking water. The estimated dose was 100 mg/kg/day, based on an assumed water consumption of 20 mL/rat/day. Subchronic toxicity studies in rats showed tolerance up to 120 mg/kg/day without significant liver effects or mortality. There are no non-rodent toxicity data.

#### Genetic toxicology:

The results of numerous genotoxicity / mutagenicity studies were positive in three of five assays. Genotoxicity was seen in the *in vitro* bacterial reverse mutation assays with metabolic activation, the sister chromatid exchange assay and the chromosomal aberration assay.

In the *in vitro* bacterial reverse mutation assays in *Salmonella typhimurium*, quinine was demonstrated as positive in the presence of metabolic activation (strain TA98), but negative in the absence of metabolic activation whereas all other strains were negative either in the presence or absence of metabolic activation.

In C3H mice, the *in vivo* micronucleus assay was positive following an oral dose of 110 mg/kg. while nonpositive findings were obtained in NMRI male and female mice following intraperitoneal or oral administrations (0.5 mmole/kg or 199 mg/kg) or following an oral dose of 110 mg/kg in Chinese hamsters. The *in vivo* sister chromatid exchange (SCE) tests showed positive results in all strains of mice tested (NMRI, C3H and C57B1), but negative in Chinese hamsters. The *In vivo* chromosomal aberration

assays were not positive in NMRI and C3H mice at oral doses of 110 mg/kg. There were non-positive genotoxicity findings in the sex-linked recessive lethal test performed in *Drosophila* at a concentration of 0.39• g/ml.

Carcinogenicity: Studies were not conducted due to the short treatment period (7 days) for malaria infection.

Reproductive toxicology:

In animal species, teratogenic effects were observed in rabbits, guinea pigs, chinchilla, and dog, but were absent in mice, and monkeys; results were equivocal in rats. Teratogenic effects were observed in rabbits (death *in utero*, degenerated auditory nerve and spiral ganglion, CNS anomalies such as anencephaly and microcephaly), guinea pigs (hemorrhage and mitochondrial change in cochlea), and chinchillas (death and growth suppression *in utero*, CNS anomalies such as anencephaly and microcephaly). There were no teratogenic findings in mice and monkeys. Embryo-fetal deaths or toxicities were observed in mice, rabbits, chinchillas and dogs, but not in rats or monkeys. The lowest observed adverse effect levels (LOAELs) for teratogenicity were approximately 200 mg/kg/day or 1600 mg/m<sup>2</sup>/day (guinea pig), 130 mg/kg/day i.m. or 1560 mg/m<sup>2</sup>/day (rabbit), and 150 mg/kg/day s.c. (chinchilla). The LOAELs were more than 1-fold of the maximum recommended human dose of 32.4 mg/kg/day (or 1199 mg/m<sup>2</sup>/day) for a 60-kg patient, on a mg/m<sup>2</sup> basis. The no-observed adverse effect levels (NOAELs) were 500 mg/kg/day or 1500 mg/m<sup>2</sup>/day (mouse), 300 mg/kg/day p.o. or 1800 mg/m<sup>2</sup>/day (rat), 50 mg/kg/day, i.m. or 1000 mg/m<sup>2</sup>/day (dog), and 200 mg/kg/day p.o. or 2400 mg/m<sup>2</sup>/day (monkey). These NOAELs are approximately 1.3x (mouse), 0.8x (dog), 1.5x (rat) and 2.0x (monkey) of the maximum recommended human dose of 32.4 mg/kg/day (or 1199 mg/m<sup>2</sup>/day) for a 60-kg patient, on a mg/m<sup>2</sup> basis. In rats, no impairment of female fertility was observed at an estimated dose of 20 mg/kg/day (or 120 mg/m<sup>2</sup>/day) of quinine sulfate administered in drinking water for a period from 2 weeks prior to mating and continuing during gestation and post-natal development. Animal data on the effect of male fertility was not available. This NOAEL is approximately 0.1x (female fertility) of the recommended human dose of 32.4 mg/kg/day (or 1199 mg/m<sup>2</sup>/day) for a 60-kg patient, on a mg/m<sup>2</sup> basis. Quinine sulfate was shown to cause adverse effects on prenatal and postnatal development in rats. In female rats receiving quinine sulfate in drinking water from 2 weeks prior to mating and continuing on during the entire pregnant and postnatal period, the pups showed impaired growth of lower body weights both at birth and during the lactating period, and delayed physical development of the teeth eruption and eyes opening during lactating period. The effects were observed at an estimated dose of 20 mg/kg/day or 120 mg/m<sup>2</sup>/day, which is approximately 0.1x of the maximum recommended human dose of 32.4 mg/kg/day or 1199 mg/m<sup>2</sup>/day for a 60-kg patient, on a mg/m<sup>2</sup> basis.

Special toxicology:

Hearing loss was demonstrated in guinea pigs (200 mg/kg) and chinchillas (150 mg/kg).

### 2.6.6.2 Single-dose toxicity

In a pharmacokinetic study (Pussard *et al.*, 2003), 3 of 6 mice died after a dose of 160 mg/kg i.p. and 2/5 died after a dose of 200 mg/kg i.p.

In the Toxic Substance List (1974) DHEW VA 77000, the minimal lethal dose following oral administration was reported as 300, 500 and 800 mg/kg in guinea pigs, rats and rabbits, respectively.

### 2.6.6.3 Repeat-dose toxicity

Repeated dose toxicity studies in the published literature consist of four 13-week (Colley *et al.*, 1989) and one 15-month (Flaks, 1978) toxicity studies in rats; no non-rodent studies were provided by the sponsor. There was a significant difference in the toxicity profile from the 13-week and 15-month studies. A high rate of mortality and adverse liver effects were observed in the chronic study (25% died within 2 months) at an estimated dose of 100 mg/kg/day. No deaths were observed in the 13-week studies (up to 200 mg/kg/day) and adverse liver effects of lesser severity were observed in one of the 13-week studies in the females of the 100 and 200 mg/kg/day dose groups. Besides the different durations of the studies, the 15-month study was conducted with quinine sulfate in drinking water of Leeds rats and the 13-week studies were conducted with quinine HCl in the diet of Sprague-Dawley rats. Any or all of these factors may have contributed to the different toxicity findings in the chronic and subchronic toxicity studies. The studies are further described below.

#### *Subchronic Toxicity Studies*

The first of the oral toxicity studies was a preliminary study, conducted in Sprague-Dawley rats (5/sex/group). Quinine HCl was given in the dietary mixture at 0, 25, 100, 200 and 250 mg/kg/day for 4 weeks. Food consumption and body weight gain were decreased in the 100, 200 and 250 mg/kg/day dose groups. A marginal impairment of food utilization efficiency was recorded at the end of the study. Females receiving 25 mg/kg/day gained less weight than the control. Increased spleen weights were observed in all treated males. In addition, the kidney and adrenal weights in all treated female groups were lower than the control group. The study supported a dose of 200 mg/kg/day as the high dose for the subsequent definitive study.

In the first 13-week definitive toxicity study, quinine HCl was given to six groups of Sprague-Dawley rats (20/sex/group) in the dietary mix at 0, 1, 10, 40, 100 or 200 mg/kg/day for 13 weeks. At the end of 13 weeks, 5 males and 5 females of each group were held for a 6-week recovery period. Clinical signs and food and water consumption were monitored throughout the study. Body weight was determined weekly. Ophthalmology, hematology and serum biochemistry were evaluated at 4- and 12-week time points. Gross and histopathology examinations were performed at the end of the study and recovery periods. There were no drug-related deaths, signs of toxicity or effects on water consumption. Body weight gains were significantly reduced at 100 and 200 mg/kg/day; but the food consumption and efficiency of food utilization were significantly reduced only at 200 mg/kg/day. Slightly elevated plasma urea levels were found at 200 mg/kg/day (males and females) and 100 mg/kg/day (males). Inorganic phosphate levels were recorded in females receiving 40 mg/kg/day and above. Slightly decreased albumin (200 mg/kg/day) and total protein and globulin (100 and 200 mg/kg/day) were reported in females only. These changes were reversible following

a 6-week recovery period. There were no eye abnormalities, or impairment of hearing function. Terminal studies showed no macroscopic abnormalities or adverse organ weight effects. In females of the 100 and 200 mg/kg/day groups, histopathological examination revealed increased incidences of moderate and/or marked periportal glycogen depletion. The incidence rates were 26% (control females), 57% (100 mg/kg/day females) and 93% (200 mg/kg/day females). The reversibility of these liver changes is unknown from the report. The no-observed-adverse-effect level (NOAEL) was estimated to be 40 mg/kg/day.

A second 13-week study was conducted in order to more accurately define the NOAEL. Sprague-Dawley rats (20/sex/group) were given quinine HCl in the dietary admixture at doses of 0, 60, 85 or 120 mg/kg/day. At the end of 13-week study, 5 males and 5 females from each group were held for a 6-week recovery period. The conduct and study parameters were identical to those of the first 13-week study described above. There were no deaths. From Week 3 onwards, slight fur loss in the 120 mg/kg/day males and females was observed. Both body weight gain and food consumption were suppressed in the males and females of the 85 and 120 mg/kg/day groups, but the effects were reversible following a 6-week recovery period. Results of ophthalmology, hematology and urinalysis examination were unremarkable. Elevated inorganic phosphate levels were found in females of the 120 mg/kg/day group and were not reversible after a 6-week of recovery period. The kidney weights of males from the 85 and 120 mg/kg/day groups were slightly higher than the control group. No liver or kidney pathology was found in this study. The NOAEL was 60 mg/kg/day.

An additional 13-week study was conducted to assess ototoxicity (Colley *et al.*, 1989). Sprague-Dawley rats received quinine in the dietary mixture at 0, 85 and 200 mg/kg/day for 13 weeks. Assessment of hearing thresholds was made using the pre-stimulus startle test method and electrocochleography. Histopathological examinations of the cochlea were performed using scanning electron microscopy of the organ of Corti and light microscopy of the *stria vascularis*. Details of the study were not given in the published report, which stated the ototoxicity study would be reported separately in more detail. The published report (Colley *et al.*, 1989) did state that all animals showed good hearing sensitivity across the sound frequency range from 2.5 to 30 kHz and no treatment-related histopathological lesions were detected. The no-effect dose was 200 mg/kg/day.

#### ***Chronic Toxicity Study***

A 15-month repeated dose toxicity study was reported in the literature (Flaks, 1978). Male Leeds rats (N=48) received 0.1% quinine sulfate in drinking water daily for 15 months. The dosage was estimated at 100 mg/kg/day based on an estimated water consumption of 20 mL/rat/day for a 200-gm rat. A group of 3 rats each were scheduled for sacrifice at 9 days, 3 weeks, 4 months, and 15 months (5 rats for the latter time point). At each time point, the same number of control rats were sacrificed and studied. Necropsy was performed on all animals that died or were sacrificed. Histopathology was conducted on the liver, kidney, and pancreas. During the first 2 months of the study, 12 rats died. At 15 months, there were 13 surviving rats, and only 1 rat survived at 20 months. This compares with no significant mortality in control animals.

Liver toxicities were observed at 4 months and included periportal glycogen depletion in all lobular areas and mild periportal fibrosis. At 15 months, glycogen loss was confined

to defined periportal zones, and small areas of cholangiofibrosis were observed in some animals. Kupffer cells showed varying degrees of lipid accumulation in the form of large cytoplasmic droplets during the study. At 15 months, binucleate hepatocytes were abundant and some cells had increased numbers of lysosomes. There was no hyperplasia or evidence of liver necrosis in the treated animals. No liver tumors were reported.

#### 2.6.6.4 Genetic toxicology

Genotoxicity of quinine has been extensively studied and published. There were positive and negative results from both *in vitro* and *in vivo* tests. Although one of the study types recommended by the ICH genotoxicity testing battery is lacking (*i.e.*, *in vitro* test in mammalian cell system), this omission is not considered significant in light of the extensive *in vivo* genotoxicity testing already conducted with quinine. The *in vivo* tests included chromosomal aberration, micronucleus and sister chromatid exchange (SCE) in rodents. In addition to the routine genotoxicity / mutagenicity tests, an evaluation of the potential for quinine to enhance ultraviolet-induced mutagenicity was performed.

#### *In Vitro Non-mammalian Cell System*

Several *in vitro* bacterial reverse mutation assays (Ames tests) of quinine have been reported. In one report (King *et al.*, 1979), the Ames test was positive in one tester strain in the presence of metabolic activation and in the other report (Munzner *et al.*, 1983), the Ames test was negative in five tester strains either in the presence or in the absence of metabolic activation.

*Salmonella*/mammalian-liver homogenate tests (Ames test) were conducted on all five *salmonella* strains (TA1535, TA100, TA1538, TA98 and TA1537) (King *et al.*, 1979). Compounds were dissolved in 30  $\mu$ L of DMSO or water. At least five concentrations of each compound were tested, usually up to 3600  $\mu$ g / plate for nontoxic and soluble compounds. S-9 was prepared from male Sprague-Dawley rats given Aroclor-1254 (500 mg/kg i.p.). Positive controls were MNNG, benzo(a)pyrene, 2-amino-anthracene and N-nitrosomorpholine. The results showed only one positive response: quinine dihydrochloride significantly increased the number of revertants in tester strain TA98 in the presence of S-9 mix. The results were negative in tester strain TA98 in the absence of S-9 mix and in TA1535, TA100, TA1538 and TA1537 tester strains irrespective of the presence or absence of S-9 mix.

In a second Ames test (Munzner *et al.*, 1983) with five *Salmonella typhimurium* tester strains (TA1535, TA100, TA1537, TA1538 and TA98), the concentrations of quinine HCl were 0.05, 0.1, 0.5, 1.0, 2.5 and 5 mg / plate. 2-Aminoanthracene was used as a positive control. The assays were performed in the presence and absence of Aroclor 1254-induced rat liver microsomes. The results were negative in all five tester strains either in the presence or in the absence of S-9.

A series of quinine concentrations in suspensions (with and without liver homogenate derived from male NMRI mice) were tested in the *E. coli*/mammalian-liver homogenate test (King *et al.*, 1979). The results showed no increases of revertants.

In a sex-linked recessive lethal test in *Drosophila melanogaster* (King *et al.*, 1979), test compounds were dissolved in 5% sucrose solution containing 2% DMSO, if necessary. The test solutions were fed to 1- to 2-day old Berlin K males for 3 days, usually at a single maximally tolerated dose (up to the LD<sub>50</sub>). The treated Berlin K males were mated

individually to 3 Basc virgin females. A mating scheme, consisting of three broods (each lasting 3 days) was used. At the end of each period the treated male was transferred to a new vial and re-mated with 3 virgin females. Usually a minimum of 1000 F<sub>1</sub> females (X-chromosome equivalents) were handled in each brood. Sex-linked recessive lethals were scored in the F<sub>2</sub> generation and confirmed in the F<sub>3</sub> generation. The results showed no lethal effects.

#### ***In Vitro Mammalian Cell System***

The sponsor's literature survey did not provide gene mutation or chromosomal aberration data from any *in vitro* mammalian cell system.

#### ***In Vivo Mammalian System***

The literature search yielded two publications on genotoxicity testing in *in vivo* mammalian systems: one testing quinine HCl in three cytogenetic tests with different endpoints in small rodents (Munzner *et al.*, 1983) and the other testing the effect of quinine diHCl in the micronucleus assay in NMRI mice (King *et al.*, 1979). SCE tests were performed in a variety of rodent studies (Munzner *et al.*, 1983). In each, 5-bromodeoxyuridine (5-BrdU) tablets were implanted in the experimental animals: 50 mg-tablet/animal with 26-hours of BrdU and 2 hours of colchicine treatment time. Four animals/group were used and 50 metaphases/animal were evaluated. A single dose of quinine HCl was given by stomach tube as an aqueous solution (0.3 mL) 2 hours after BrdU implantation. The dose was 110 mg/kg (p.o.) in Chinese hamsters and NMRI mice. Cyclophosphamide (10 mg/kg i.p.) served as the positive control. Because of positive results in the NMRI mice, the study was repeated in two other inbred strains of mice (*i.e.*, C3H and C57B1) using three dosing levels: 55, 75 and 110 mg/kg. Quinine HCl was negative in the SCE test in Chinese hamsters, but was positive in all strains of mice tested, *i.e.*, NMRI mice, C3H mice and C57B1 mice, with a dose-related response in C57B1 mice.

Munzner and colleagues (Munzner *et al.*, 1983) also reported a mouse micronucleus test that was performed in NMRI and C3H mice and Chinese hamsters following a single oral administration of quinine HCl (110 mg/kg). Thirty hours post-dose, the bone marrow cells were harvested. Six animals/group were used and 1000 polychromatic erythrocytes/animal were evaluated for micronuclei. Cyclophosphamide (20 mg/kg i.p.) was the positive control. Quinine HCl was negative in NMRI mice and Chinese hamsters, but induced micronuclei in marrow cells of C3H mice.

King and colleagues (King *et al.*, 1979) reported a micronucleus test performed in male and female NMRI mice (4/sex/group). One control and three dosed groups were included. Quinine diHCl (0.50 mmole/kg or 199 mg/kg) was given either *via* i.p. or p.o. twice, over a 24-hour period. Six hours following the second dose, bone marrow smears were prepared and stained. A total of 1000 polychromatic erythrocytes were analyzed for each animal. The results indicated that quinine diHCl did not increase micronuclei in the bone marrow of NMRI mice.

Thus, following either oral or i.p. administration of quinine HCl to NMRI mice, micronucleus tests were negative when conducted by two different investigators. Chromosome aberration tests were conducted in NMRI mice, C3H mice, and Chinese hamsters (Munzner *et al.*, 1983). Quinine HCl was administered as a single oral dose

(110 mg/kg), as in the SCE test above, with a 24-hour quinine and 2-hour colchicine treatment. Cyclophosphamide 64 mg p.o. was included as a positive control. Six animals/group were studied and 300 metaphases/animal were scored for structural aberration (only gaps and chromatid breaks). Quinine HCl did not induce chromosomal aberrations in any of the species / strains studied.

#### ***Other Systems***

An additional mutagenicity assay investigated the potential for a number of antimalarial drugs to enhance UV-induced mutagenicity (Sideropoulos *et al.*, 1980). Following irradiation of specific tester strains with UV radiation (40 to 60 ergs/mm<sup>2</sup>), chemicals that interfere with repair of UV-induced pre-mutational lesions significantly enhance the frequency of mutation to streptomycin-resistance.

The study was performed in radiation-resistant *E. coli* strain B/r and WP2 her+ and her-, which were exposed to a non-lethal dose of UV light. Antimalarial drugs tested were quinine, quinine HCl, quinine hydrobromide, primaquine diphosphate, chloroquine and quinacrine dihydrochloride; quinine was included in the Brain Heart Infusion (BHI) at a concentration of 50 µg/mL. After 3.5 hours of incubation on BHI with and without the chemicals, the membranes were transferred to BHI agar plates containing dihydrostreptomycin sulfate. The plates were incubated for 72 hours at 37°C for selection of the mutant. The progeny of resistant mutants that produced colonies on the lawn of the drug-sensitive cells were counted.

All antimalarial drugs increased mutant frequency in the presence of UV light. For quinine, the synergistic activity ranged from 6- to 12-fold. None were mutagenic without UV light.

#### **2.6.6.5 Carcinogenicity**

Carcinogenicity studies were not conducted for this application as the product will be administered for acute clinical exposure.

#### **2.6.6.6 Reproductive and developmental toxicology**

Quinine is a known human teratogen, an observation primarily based on very high doses (up to 30 g) used as an abortifacient. Teratogenic effects have also been shown in some animal species such as rabbits, guinea pigs and chinchilla, but not in other species such as mice, rats, dogs and monkeys. In humans, malformations include auditory nerve hypoplasia leading to deafness (accounting for 50% of malformations), limb anomalies, visceral defects, and visual changes. In animals, malformations included hemorrhage in cochlea (guinea pig), central nervous system anomalies, such as anencephaly and microcephaly (rabbit and chinchilla). The teratogenic doses were 1300 mg/animal (guinea pig), 130 mg/kg/day i.m. (rabbit), and 150 mg/kg/day s.c. (chinchilla). No malformations were observed in mice (up to 500 mg/kg/day), rats (up to 300 mg/kg/day, p.o.), dogs (up to 50 mg/kg/day, i.m.) and monkeys (up to 200 mg/kg/day, p.o.). Embryo-fetal toxicity, as evidenced by lethality to embryos and fetuses, was observed in rabbits at 100 mg/kg i.m. and in dogs at 15 to 50 mg/kg i.m., but not in rats (300 mg/kg, p.o.). The available data on reproductive and developmental toxicity, considered as most relevant, are summarized in the discussions and table below.

***Effects on Fertility and Early Embryonic Development***

The sponsor's literature survey did not identify any fertility study in animals *per se*. But quinine did not appear to impair female fertility in rats. In a study on effects of prenatal and postnatal development (Lapointe *et al.*, 1979), female rats received quinine in drinking water 2 weeks prior to mating with untreated male rats; treatment of the female rats continued throughout mating, gestation and lactation /nursing period up to weaning on Day 21 of post parturition. The pregnancy rate, litter size and duration of gestation showed no treatment-related effects. In addition, two 3-month subchronic toxicity studies showed no adverse effects on the reproductive organs of male and female rats, suggesting that in both male and female rats, reproductive function, such as fertility, was not likely to be affected.

***Effects on Embryo-fetal Development***

Data on effects of embryo-fetal development are available in mice, rats, guinea pigs, rabbits, dogs, chinchillas and monkeys. Teratogenic effects were shown in rabbits, guinea pigs and dogs, but not in mice, and monkeys. Results are equivocal in rats. One of the references used in this evaluation was published in French and was translated (Savini *et al.*, 1971). Embryo-fetal toxicity (embryoletality) was observed in rabbits at 100 mg/kg i.m. and dogs at 15 to 50 mg/kg i.m., but not in rats (300 mg/kg, p.o.).

Another study in rats also showed a lack of teratogenic effects at doses up to 200 mg/kg/day p.o. (Colley *et al.*, 1989). The effect of quinine HCl on embryo-fetal development in Sprague-Dawley rats (25/group) was conducted in rats receiving quinine HCl by gavage at 0, 50, 100 or 200 mg/kg/day during Days 6 to 18 of gestation. Clinical signs, body weight and food consumption were monitored. On Day 20 of gestation, the dams were sacrificed and all litter parameters recorded. Half of the fetuses from each litter were preserved for visceral examination by serial sectioning and the other half for skeletal examination following the alizarin-red-staining procedure. Structural changes among offspring were classified in order of severity and/or rarity, as malformations, anomalies and variants. Litter data were analyzed statistically using non-parametric methods. Dose-related signs were salivation and localized fur loss, observed from 100 to 200 mg/kg/day. During the dosing period, there was increased water consumption and a slight reduction in weight gain in these two groups. There were no significant effects on malformations or pre- and post-implantation losses. At 200 mg/kg/day, however, slight but statistically significant decreases of fetal and litter weights were reported, together with the increased incidences of visceral and skeletal anomalies and variant sternbrae. These effects were probably secondary to quinine-related maternal toxicity such as overt signs and slight reduction of weight gain in this group.

The effects of quinine HCl on embryo-fetal development were investigated in Japanese monkeys (*Macaca fuscata*) and rhesus monkeys (*Macaca mulatta*) in Tanimura (1972). No teratogenic effects were observed in either species up to 200 mg/kg/day. The menstrual cycles of each female were recorded. Japanese female monkeys were given clomiphene citrate orally, daily dosage of 25 mg between the 5<sup>th</sup> to 7<sup>th</sup> day of the cycle, then housed with an adult male for 48 hours starting on the 12<sup>th</sup> day of the cycle. Rhesus female monkeys received no drug and the determination for the starting day of the mating was based on external signs of estrus (swollen sexual skin, increase of viscous cervical

fluid and vaginal smear with increased eosinophilic leukocytes). The middle of the mating period was considered Day 0 of gestation for both species. Diagnosis of pregnancy was made by serum chorionic gonadotropin assay.

Pregnant animals of each species were divided into four groups and animals of each group were treated as follows:

Group 1: Thalidomide, orally at 20 mg/kg on 24, 25 and 26 consecutive gestation days.

Group 2: Quinine HCl, orally at daily dosage of 20, 100 and 200 mg/kg on 27, 28 and 29 consecutive gestation days.

Groups 3 / 4 Given aminopterin and acetylsalicylic acid, respectively. Not pertinent.

Two control groups: One without treatment and the other receiving carboxymethyl cellulose daily at 25 mg/kg on 24, 25 and 26 consecutive gestation days.

All females underwent Caesarean section on Days 58 to 62 of gestation and their conceptuses (fetuses and membranes *in toto*) were recovered. Examination was made for abnormal findings in the uterine cavity. Live fetuses were weighed, sexed and examined for external development, following which internal anomalies (brain, thoracic and abdominal organs) were evaluated by means of micro-dissection. Bodies were cleared and examined for skeletal anomalies. Quinine HCl, at doses up to 200 mg/kg/day, was not teratogenic either in Japanese or rhesus monkeys. Two of 6 treated monkeys had retroplacental hematoma and/or yellowish amniotic fluid, suggesting potential abortive effects of quinine in pregnant monkeys. Thalidomide (20 mg/kg/day) caused typical limb malformations as well as additional skeletal defects in all fetuses of both Japanese and rhesus monkeys.

#### ***Effects on Prenatal and Postnatal Development Including Maternal Function***

A study was undertaken to determine the effect of saccharin, a sweetener, and quinine sulfate, a bitter substance, on postnatal development in rats (Lapointe *et al.*, 1979). The results showed that postnatal physical growth was impaired in quinine-treated pups. Three groups of female Sprague-Dawley rats (5/group) received quinine sulfate (0.25 mg/mL), saccharin (0.4 mg/mL) or control (tap water) from 2 weeks prior to mating and continuing during pregnancy and the postnatal period. The treated females were mated with untreated males. Maternal weight, food and liquid intakes were measured during the pregnancy and lactating period. Each delivered litter was reduced at birth to 8 pups (4 males and 4 females). The remaining neonates (up to 8) were used for skeletal examination (alizarin procedure). Selected gross behavioral studies, conducted up to weaning (21 days of age) included surface righting reflex, sensory organ evolution such as eyes opening, ears unfolding and opening, and two features of physical evolution, *i.e.*, teeth eruption and fur development.

Quinine-drinking pregnant dams demonstrated a decreased ingestion of liquid whereas saccharin-drinking pregnant dams exhibited polydipsia. There were no significant effects of treatment on the food intake, body weight gain, and pregnancy rate, length of gestation days or litter size. Compared to the control group, the birth weight of both male and female neonates of the quinine group was significantly lower. Gross external anomalies were observed in 2 quinine pups: 1 with syndactylia in the right forelimb whereas an anophthalmia involving the right eye of the other pup was present at birth but became

more evident in the 18-day old rat (time of normal eyes opening). The result was significant as 5% of the pups showed these malformations. There were no signs of dysmorphogenesis, either spontaneous or drug-induced, in several hundred rat pups studied. Quinine-exposed pups exhibited a statistically significant delay in teeth eruption and eye opening. Postnatal physical growth was impaired in quinine-treated pups. These findings corroborated those in guinea pigs, rabbits and human baby, reported by Savini and colleagues (Savini *et al.*, 1971).

The dosage of quinine is estimated at 20 mg/kg/day, assuming each rat, weighing 250 g, drank 20 mL water per day. The study showed quinine affected prenatal and postnatal development in rats with lower body weights both at birth and during lactating period, and delayed teeth eruption and eye opening. The estimated LOAEL was 20 mg/kg/day.

#### 2.6.6.7 Local tolerance

Not conducted

#### 2.6.6.8 Special toxicology studies

Quinine-induced ototoxicity has been shown in animal species and appears to be multifactorial. The available nonclinical data indicate that mechanisms may include vasoconstriction and decreased cochlear blood flow as measured by laser Doppler flowmetry, motion photography and histological studies. Reversible alterations of outer hair cells also appear to play an important role, as demonstrated by histology, electron microscopy, isolated hair cell studies, and cochlear potential evaluations. The roles of prostaglandins as well as antagonism of calcium-dependent potassium channels in quinine-induced ototoxicity have not been elucidated.

In a guinea pig study (Alván *et al.*, 1989), hearing thresholds increased with increasing quinine blood concentration when intravenously infused at doses of 5 to 50 mg/kg, with 1 to 12 doses per animal. Plasma concentrations ranged from 7.8 to 31.3  $\mu\text{g/mL}$ ; the "no effect" level was estimated at 5.5  $\mu\text{g/mL}$ . Loss of outer hair cells has been associated with hearing loss in guinea pigs (Ruedi *et al.*, 1952 and Covell, 1938 as reviewed by Jung *et al.*, 1993).

Hearing loss and recovery (tested by auditory brainstem response) was measured *in vivo* following intramuscular (i.m.) injection of quinine HCl (150 mg/kg) to chinchillas (6 ears tested) and changes in cochlear blood measured when quinine (5  $\mu\text{g}$ ) was applied topically to the round window membrane (RWM) of 5 ears (Lee *et al.*, 1992). The cochlea were harvested at 2 hours when hearing loss was maximal. Scanning electron microscopy revealed no loss of hair cells. Mean perilymph levels of quinine were confirmed to be comparable by both routes (approximately 350 to 440  $\mu\text{g/mL}$ ). *In vivo*, a 20-dB hearing loss occurred that peaked 8 hours post-dose and returned to normal by 18 hours. Laser Doppler flowmetry on 5 animals in the RWM experiment demonstrated an initial decrease in blood flow, which then reverses, paralleling the pattern of observed hearing loss.

#### 2.6.6.9 DISCUSSION AND CONCLUSIONS

Quinine is an antimalarial agent that has been used for hundreds of years. Quinine acts primarily as a blood schizontocide with little effect on sporozoites or pre-erythrocytic forms of malarial parasites. The alkaloid is also gametocidal for *P. vivax* and

*P. malariae*, but not for *P. falciparum*. Because of this spectrum of anti-malarial activity, quinine is not used for prophylaxis.

Quinine is a stereoisomer of the antiarrhythmic drug, quinidine. Available nonclinical data have shown similar cardiovascular properties for these two compounds, but quinine is generally less potent. Quinine causes hypotension, increased heart rate and myocardial contractility in dogs and other animal species. Quinine-induced hypotension appears to be due to peripheral vasodilation. The vasodilative effect of quinine is probably caused by a direct relaxing or depressive effect on the smooth muscle of blood vessel and/or an  $\alpha$ -adrenergic antagonizing effect. Quinine, like quinidine, blocks HERG channels and delayed rectifier K<sup>+</sup> currents, with a relative potency that is approximately 10-fold less than that of quinidine. Quinine interacts with open K<sup>+</sup> channels and inactivates Ca<sup>2+</sup> channels, blocks potassium and sodium conductances and probably induces opening of hemi-gap junctional channels. As an anti-arrhythmic agent, quinidine prolongs both refractoriness and conduction time. Quinine, however, prolongs only the conduction time but not refractoriness. As an antiarrhythmic agent, quinidine acts via slowing conduction and reducing automaticity in all parts of the heart, with increase of the effective refractory period. Quinidine prolongs the QT interval in a dose-related fashion, which may lead to increased ventricular automaticity and polymorphic ventricular tachycardias, including *torsades de pointes*. Based on the comparative inhibition data of delayed rectifier K<sup>+</sup> currents, however, the propensity for quinine-induced QT prolongation is about an order of magnitude less than that of quinidine. Quinine increases the refractory period of skeletal muscle by direct action on the muscle fiber and the distribution of calcium within the muscle fiber, thereby diminishing the response to tetanic stimulation. It also decreases the excitability of the motor end-plate region, reducing the responses to repetitive nerve stimulation and to acetylcholine. Quinine can antagonize the action of physostigmine on skeletal muscle as effectively as curare.

Quinine-induced ototoxicity was observed in animal species and appears due to several factors. The mechanisms may include vasoconstriction and decreases in cochlear blood flow. Reversible alterations of outer hair cells also appear to play an important role, as demonstrated by histology, electron microscopy, isolated hair cell studies, and cochlear potential evaluations. In guinea pigs, hearing thresholds increased with quinine blood concentrations. In chinchilla the perilymph levels were 356 to 440 ng/mL at peak hearing loss. In a repeated-dose toxicity study in rats, however, no signs of ototoxicity were observed. Following 13 weeks of oral treatment with quinine HCl at doses up to 200 mg/kg/day to rats, all animals showed good hearing sensitivity across the sound frequency ranges of 2.5 to 30 kHz and no treatment-related histopathological lesions in the organ of Corti or stria vascularis.

Quinine is absorbed following oral administration, concentrating in erythrocytes and distributing to the major organs of liver, heart, lung kidneys and muscle; distribution is increased in infected animals. Brain distribution is low and unaffected by infection. Placental transfer of quinine occurs; in sheep, the fetal exposure level is less than 10% of the maternal exposure. The drug is rapidly excreted from the body, with plasma half-lives in mice, rats and dogs of approximately 1, 13 to 16, and 7 hours, respectively. In mice, clearance is decreased and exposure is increased in infected animals. Quinine undergoes extensive metabolism in the liver in all animal species

tested. The major metabolite is 3-hydroxyquinine; there are other unidentified metabolites. *In vitro* studies showed CYP450 3A isozyme is the responsible enzyme in animal species and humans. Unchanged drug accounts for approximately 10 to 20% of the administered dose in urine in the first 24 hours and half-life is prolonged in models of acute renal failure. Urine and feces are routes of excretion.

Repeated dose toxicity studies have been performed in rats. The chronic toxicity of quinine sulfate include high rate of mortality and adverse liver effects. The duration-related liver toxicities include periportal glycogen depletion in all lobular areas and mild periportal fibrosis, small areas of cholangiofibrosis, Kupffer cells with varying degrees of lipid accumulation in the form of large cytoplasmic droplets, abundant binucleate hepatocytes, and increased numbers of lysosomes. There was no hyperplasia or evidence of liver necrosis in the treated animals. No liver tumors were reported. The study was conducted in male Leeds rats with quinine sulfate in drinking water. The estimated dose was 100 mg/kg/day, based on an assumed water consumption of 20 mL/rat/day.

Subchronic toxicity studies in rats showed tolerance up to 120 mg/kg/day without significant liver effects or mortality. There are no non-rodent toxicity data.

The results of numerous genotoxicity / mutagenicity studies were positive in three of five assays. Genotoxicity was seen in the *in vitro* bacterial reverse mutation assays with metabolic activation, the sister chromatid exchange assay and the chromosomal aberration assay .

In the *in vitro* bacterial reverse mutation assays in *Salmonella typhimurium*, quinine was demonstrated as positive in the presence of metabolic activation (strain TA98), but negative in the absence of metabolic activation whereas all other strains were negative either in the presence or absence of metabolic activation.

In C3H mice, the *in vivo* micronucleus assay was positive following an oral dose of 110 mg/kg. while nonpositive findings were obtained in NMRI male and female mice following intraperitoneal or oral administrations (0.5 mmole/kg or 199 mg/kg) or following an oral dose of 110 mg/kg in Chinese hamsters. The *in vivo* sister chromatid exchange (SCE) tests showed positive results in all strains of mice tested (NMRI, C3H and C57B1), but negative in Chinese hamsters. The *In vivo* chromosomal aberration assays were not positive in NMRI and C3H mice at oral doses of 110 mg/kg. There were non-positive genotoxicity findings in the sex-linked recessive lethal test performed in *Drosophila* at a concentration of 0.39• g/ml.

The carcinogenicity of quinine was not studied in laboratory animals.

In animal species, teratogenic effects were observed in rabbits, guinea pigs, chinchilla, and dog, but were absent in mice, and monkeys; results were equivocal in rats.

Teratogenic effects were observed in rabbits (death *in utero*, degenerated auditory nerve and spiral ganglion, CNS anomalies such as anencephaly and microcephaly), guinea pigs (hemorrhage and mitochondrial change in cochlea), and chinchillas (death and growth suppression *in utero*, CNS anomalies such as anencephaly and microcephaly). There were no teratogenic findings in mice and monkeys. Embryo-fetal deaths or toxicities were observed in mice, rabbits, chinchillas and dogs, but not in rats or monkeys.

The lowest observed adverse effect levels (LOAELs) for teratogenicity were approximately 200 mg/kg/day or 1600 mg/m<sup>2</sup>/day (guinea pig), 130 mg/kg/day i.m. or 1560 mg/m<sup>2</sup>/day (rabbit), and 150 mg/kg/day s.c. (chinchilla). The LOAELs were more than 1-fold of the maximum recommended human dose of 32.4 mg/kg/day (or 1199

mg/m<sup>2</sup>/day) for a 60-kg patient, on a mg/m<sup>2</sup> basis. The no-observed adverse effect levels (NOAELs) were 500 mg/kg/day or 1500 mg/m<sup>2</sup>/day (mouse), 300 mg/kg/day p.o. or 1800 mg/m<sup>2</sup>/day (rat), 50 mg/kg/day, i.m. or 1000 mg/m<sup>2</sup>/day (dog), and 200 mg/kg/day p.o. or 2400 mg/m<sup>2</sup>/day (monkey). These NOAELs are approximately 1.3x (mouse), 0.8x (dog), 1.5x (rat) and 2.0x (monkey) of the maximum recommended human dose of 32.4 mg/kg/day (or 1199 mg/m<sup>2</sup>/day) for a 60-kg patient, on a mg/m<sup>2</sup> basis.

In rats, no impairment of female fertility was observed at an estimated dose of 20 mg/kg/day (or 120 mg/m<sup>2</sup>/day) of quinine sulfate administered in drinking water for a period from 2 weeks prior to mating and continuing during gestation and post-natal development. Animal data on the effect of male fertility was not available. This NOAEL is approximately 0.1x(female fertility) of the recommended human dose of 32.4 mg/kg/day (or 1199 mg/m<sup>2</sup>/day) for a 60-kg patient, on a mg/m<sup>2</sup> basis.

Quinine sulfate was shown to cause adverse effects on prenatal and postnatal development in rats. In female rats receiving quinine sulfate in drinking water from 2 weeks prior to mating and continuing on during the entire pregnant and postnatal period, the pups showed impaired growth of lower body weights both at birth and during the lactating period, and delayed physical development of the teeth eruption and eyes opening during lactating period. The effects were observed at an estimated dose of 20 mg/kg/day or 120 mg/m<sup>2</sup>/day, which is approximately 0.1x of the maximum recommended human dose of 32.4 mg/kg/day or 1199 mg/m<sup>2</sup>/day for a 60-kg patient, on a mg/m<sup>2</sup> basis.

#### 2.6.6.10 TOXICOLOGY TABULATED SUMMARY

species	Duration of dosing	NOAEL (mg/kg)	NOAEL (HED mg/kg)	Multiple of human dose (32.4 mg/kg)
rat	13 weeks	40 mg/kg	6.4	0.20
Rat	13 weeks	60 mg/kg	9.7	0.30

Table 5.13  
Summary of Repeated Dose Toxicology Studies of Quinine

Reference	Species/ Strain	N / Sex /Group	Dose (mg/kg/day) / Duration / Route	Parameters	Results / Observations
<u>Colley et al., 1989</u>	Sprague-Dawley rats	5	0 or 25, 100, 200 or 250  13 weeks  oral (diet)	A preliminary study to select doses for definitive study.  Not clearly stated, but included body weight, food consumption, and organ weight.	Food consumption and weight gains were lower at 100-250 mg/kg/day. Food utilization efficiency impaired at the end of study. Females receiving 25 mg/kg/day gained less weight. Increased spleen weight in all treated males. Decreased kidney and adrenal weights in all treated female groups.  200 mg/kg/day was selected as high dose for definite 13-week study
<u>Colley et al., 1989</u>	Sprague-Dawley rats	20  5 from above. 6-week recovery	0 or 1, 10, 40, 100 or 200  13 weeks  oral (diet)	Clinical signs, body weight, food and water consumption, ophthalmoscopy, hematology, serum biochemistry at 4 and 12 weeks, organ weight (adrenals, brain, heart, kidney, liver, thyroid, pituitary, spleen, testes, ovary, uterus) and "a wide range of tissues" for histopathology.	No drug-related death or overt signs of toxicity. Food consumption and weight gains were lower at 100-200 mg/kg/day (males and females). Water consumption was not affected. Food utilization efficiency impaired at 200 mg/kg/day. Increased plasma urea at 200 mg/kg/day (males and females) and 100 mg/kg/day (males). Higher phosphorus levels were observed from 40 mg/kg/day (females). The albumin levels were slightly lower at 200 mg/kg/day (females) and lower total protein and globulin at 100-200 mg/kg/day (females). Organ weight and gross pathology were unremarkable. Histopathology showed increased incidence of moderate and/or marked periportal glycogen depletion in the liver of 100 and 200 mg/kg/day females; the incidence rates were 26% (control), 57% (100 mg/kg/day) and 97% (200 mg/kg/day).  Ophthalmological examination showed no adverse effects; hearing was not impaired.  Effects on body weight and serum biochemistry were reversible following a 6-week withdrawal period. The reversibility of liver effect (glycogen depletion) was not stated in the paper.  NOAEL is estimated at 40 mg/kg/day for both males and females.  LOAEL (depletion of hepatic glycogen, body weight, food consumption, serum biochemistry) is estimated at 100 mg/kg/day for both males and females.

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Reference	Species/ Strain	N / Sex /Group	Dose (mg/kg/day) / Duration / Route	Parameters	Results / Observations
<u>Colley et al., 1989</u>	Sprague-Dawley rats	20  5 from above: 6-week recovery	0, 60, 85 or 120  13 weeks  oral (diet)  Conducted to narrowly define NOAEL	Same as above.	No deaths were recorded; slight loss of fur from Week 3 onward at 120 mg/kg/day (males and females). Food consumption and weight gain were lower at 85 and 120 mg/kg/day (males and females). Higher phosphorus levels were observed at 120 mg/kg/day (females). Urinalysis was unremarkable. Kidney weights of 85 and 120 mg/kg/day males were minimally increased. Gross examination and histopathology were unremarkable; no liver or kidney effects.  Ophthalmological examination showed no adverse effects.  Following a 6-week withdrawal period, effects on body weight and food consumption were reversible, but the elevated phosphorus levels tended to persist.  NOAEL is estimated at 60 mg/kg/day for both males and females.  LOAEL (Body weight and food consumption) is estimated at 85 mg/kg/day for both males and females.
<u>Flaks, 1978</u>	Albino Leads strain rats	48 male total  Groups of 3 rats at interim sacrifices; 5 in final group.  Control rats used	Oral  0.1% quinine sulfate in drinking water, daily for 15 months.  Interim sacrifices at 9 days, 3 weeks, 4 months (control and experimental)	Histopathology (hepatic, renal, pancreatic tissues) and EM studies (hepatic tissues)	By 2 mo.: 12 deaths By 12 mo.: additional 15 deaths (21 survived) By 15 mo.: additional 8 deaths (13 survived) At 20 mo.: additional 12 deaths (1 survived) No tumors; no hyperplasia, no liver necrosis. Liver: duration related effects. Periportal glycogen depletion- from 4 mo. From 4-15 mo., periportal fibrosis to cholangiofibrosis in some animals. Kupffer cells: varying degrees of lipid accumulation in the form of large cytoplasmic droplets. By 15 mo., binucleate hepatocytes were abundant with increased numbers of lysosomes in some cells. Cytoplasm vacuoles might represent enlarged or swollen mitochondria seen. Bile canaliculi enlarged and dilated. Kidneys: minimal tubular cell necrosis in renal cortex of some rats during the early part of the study. Control rats: normal liver and kidney.

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Table 5.14  
Summary of Mutagenicity Studies of Quinine

Reference	Species/ Strain	N / Sex /Group	Dose or Concentration	Parameters	Results / Observations		
<u>King et al.</u> 1979	<i>Salmonella typhimurium</i> TA1535, TA100, TA1538, TA98 and TA1537	N / A	Five concentrations: up to 3600 µg/plate	Ames test (No. of revertants = S-9 mix)	Positive: TA98 in the presence of S-9. Negative: TA100, TA1535, TA1537 and TA1538 = S-9 mix. TA98 - S-9 mix.		
	<i>E. coli</i>	N / A	1.57 µg/mL (+/- S-9 mix)	No. of revertants = S-9 mix (from NMRI mice)	Negative: <i>E. coli</i> = S-9 mix		
	<i>Drosophila melanogaster</i>	N / A	0.39 µg/mL	Recessive lethal mutation	Negative: Sex-linked recessive lethals in <i>Drosophila</i>		
	Micronucleus test: Mice	4 M	3 doses + control	Micronuclei / 1000 polychromatic erythrocytes	Negative: up to 2 x 0.5 mmole/kg or 2 x 199 mg/kg, i.p. or p.o.		
<u>Munzner et al.</u> 1983	Ames test <i>Salmonella typhimurium</i> TA1535, TA100, TA1538, TA98 and TA1537	N / A	0, 0.05, 0.1, 0.5, 1.0, 2.5 and 5 mg/plate	No. of revertants = S-9 mix (from Sprague - Dawley rats)	Negative: TA98, TA100, TA1535, TA1537 and TA1538 = S-9 mix		
	NMRI mice Chinese hamsters	4	110 mg/kg p.o., vehicle and (+) Control (C): (Cyclophosphamide 10 mg/kg i.p.)	Sister Chromatid Exchange (SCE) (50 bone marrow cell metaphases/ mouse analyzed)	NMRI mice: Positive Vehicle: 4.54 ± 0.08 Quinine: 5.17 ± 0.13* (-) C: 20.88 ± 1.32 Chinese hamster: Negative		
	Repeat Assay: C3H mice C57B1/6J mice		55, 75, 110 mg/kg	Sister Chromatid Exchange (SCE) (50 bone marrow cell metaphases/ mouse analyzed)	C3H mice: Positive Vehicle: 6.95 ± 0.31 55 mg/kg: 7.39 ± 0.38 75 mg/kg: 8.01 ± 0.35 110 mg/kg: 8.97 ± 0.07* C57B1 mice: Positive Vehicle: 6.83 ± 0.11 55 mg/kg: 7.31 ± 0.27 75 mg/kg: 8.25 ± 0.24* 110 mg/kg: 9.28 ± 0.57*		
	NMRI mice C3H mice Chinese hamsters	6	110 mg/kg p.o. (-) Control (C): (Cyclophosphamide 20- mg/kg i.p.)	Micronucleus test (micronuclei per 1000 polychromatic erythrocytes)	NMRI mice: Negative. C3H mice: Positive. Vehicle: 3.66 110 mg/kg: 7.60** Chinese hamster: Negative.		
	NMRI mice C3H mice Chinese hamsters	6	110 mg/kg p.o. (-) Control (C): (Cyclophosphamide 64 mg/kg p.o.)	Chromosome aberrations (300 metaphases / animal for structural aberrations).	Data as % chromatid breaks. (Only gaps and chromatid breaks occurred). NMRI mice: Negative. C3H mice: Negative. Chinese hamsters: Negative.		
<u>Sideropoulos et al.</u> 1980	Radiation-resistant <i>E. coli</i> Strain B/r and WP2 lex- try	50 µg/mL	Quinine (Q), Quinine HCl (QHCl), Quinine HBr (QHBr)	Frequency of mutations to streptomycin resistance	#	Fold Increase	
					C+UV	41	-
					Q+UV	192	11.9
					QHCl+UV	240	5.8
QHBr+UV	132	4.7					

N / A: Not Applicable

\*t-test, p &lt; 0.001

\*\*Wilcoxon rank test, p &lt; 0.01

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## Reproductive toxicity NOAELs in animals in comparison to Proposed Human Dose

Animal	NOAEL (mg/kg/day)	Human Equivalent Dose (mg/kg/day)	NOAEL (mg/m2/day)	Estimated X Human Dose (1199 mg/m2/day)
Mouse	500	42	1500	1.3 X
Rat	300	50	1800	1.5 X
Dog	50	28	1000	0.8 X
Monkey	200	65	2400	2.0 X

Table 5.16

## Summary of Maternal and Fetal Data in a Segment II Study of Quinine in the Rat

Observation	Mean Value at Dosage (mg/kg/day)			
	0	50	100	200
Water Consumption (g/rat/day) Days 6-16	36.2	38.8	66.0	78.3
Weight Gain (g) Days 6-10 Days 10-14	23.0 24.4	22.3 22.5	16.7 25.4	18.0 21.1
Fetal Weight (g)	3.51	3.55	3.54	3.30*
Fetal Change (%) malformations visceral anomalies skeletal anomalies variant sternbrae	0.8 3.2 10.8 52.9	0.4 1.5 19.2 44.3	0.0 2.8 12.8 61.9	1.8 11.0 24.0 71.0*

\*Source: Collev *et al.*, 1989

P &lt; 0.05 in comparison with control, Kruskal-Wallis test.

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Table 5.15

## Effects of Quinine Administered During Pregnancy on Mammalian Offspring

Author(s)	Species	Dose (mg/kg/day) Route*	Time of treatment	Developmental anomalies
Covell (1936)	Guinea pig	200	3 weeks	Mitochondrial change in cochlea
West (1938)	Rabbit	65-325 mg/head	term?	Degenerated auditory nerve and spiral ganglion
Mosher (1938)	Guinea pig	1300 mg/head	Various	Hemorrhage in cochlea
Belkina (1958)	Rabbit	130	4-8 days during the first semester of pregnancy	Death <i>in utero</i> . CNS anomalies such as anencephaly and microcephaly
Klосovskii (1963)	Chinchilla	90-150 s.c.	1-8 days during the 4 <sup>th</sup> to 14 <sup>th</sup> days of gestation	Death and growth suppression <i>in utero</i> . CNS anomalies such as anencephaly and microcephaly
Neuweiler & Richter (1964)	Rat	50	7-14 days of gestation	No malformation
Savini <i>et al.</i> (1971)	Rat	less than 300 p.o.	7-18 days of gestation	No embryotoxicity
Savini <i>et al.</i> (1971)	Rabbit	100 i.m.	10-28 days of gestation	Death of all embryos <i>in utero</i>
Savini <i>et al.</i> (1971)	Dog	15-50 i.m.	18-48 days of gestation	Death <i>in utero</i>
Tanimura & Lee (unpubl.)	Mouse	125-500	6-12 days of gestation	Lethal and growth suppression. No malformations
Tanimura (1972)	Monkey	20-200 p.o.	27-29 days of gestation	No teratogenicity

Source: Tanimura, 1972

\*Routes in some animal species are not available

Table 3.1 /  
Summary of Reproductive and Developmental Toxicity Studies of Quinine

Reference	Species/ Strain	N / Sex / Group	Dose (mg/kg/day) or Concentration/ Route	Parameters	Results / Observations
<u>Tanimura, 1972</u>	Japanese monkey ( <i>Macaca fuscata</i> )  Rhesus monkey ( <i>Macaca mulatta</i> )  Female monkeys were mated with males on 12 <sup>th</sup> day of cycle.	5 treated  1 untreated control  1 vehicle control  1 Thalidomide control	<u>Quinine HCl</u> 20 (1 Japanese and 3 Rhesus monkeys), 100 (1 Rhesus monkey) 200 (1 Rhesus monkey) Days on 27, 28 & 29 of gestation  <u>Control:</u> untreated (1 rhesus monkey)  <u>Vehicle control:</u> carboxymethyl cellulose (CMC) 25 mg/kg on 24, 25 & 26 consecutive gestation days.  Oral	Caesarean section on Days 58 and 62 of gestation; conceptuses (fetus and membranes <i>in toto</i> ) were recovered.  Uterine cavity examined, fetuses weighed, sexed, conducted external, visceral (thoracic and abdominal organs) & skeletal examination.	<u>Quinine HCl, 6 fetuses total:</u> No external, visceral and skeletal anomalies in monkeys receiving 20, 100 & 200 mg/kg/day. Lower body weight and smaller crown- rump length in 200 mg/kg/day group than controls.  <u>Fetal membranes:</u> 2 Rhesus monkeys: 1 had yellow amniotic fluid and retroplacental hematoma and the other had retroplacental hematoma, suggesting potential abortive effects in pregnant monkeys.  <u>Untreated and CMC controls,</u> <u>1 Fetus each:</u> No external, visceral or skeletal anomalies. Body weight was 15.9 g (untreated) and 11.8 g (CMC). Crown-rump length: was 60 mm (each fetus). Fetal membranes were normal.  Thalidomide included as a control group and evidenced the expected anomalies.

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Table 5.17  
Summary of Reproductive and Developmental Toxicity Studies of Quinine (continued)

Reference	Species/ Strain	N / Sex / Group	Dose (mg/kg/day) or Concentration/ Route	Parameters	Results / Observations
<u>Lancinte et al., 1979</u>	Sprague-Dawley rats	3 groups  5F each	<u>Quinine sulfate (0.22 mg/mL)</u>  Orally via drinking water during the entire experimental period. Following a 2-week treatment, female rats were mated with untreated males.  Vehicle control Saccharin control	<u>Dams:</u> Body weight, food and liquid consumption.  <u>Pups:</u> culled to 8 (4M + 4F) for postnatal study; the remaining pups for skeletal (alizarin stain) and EM studies.  <u>Postnatal development:</u> <u>Surface righting reflex.</u>  <u>Sensory organs:</u> (eye opening, ears unfolding and opening).  <u>Physical development:</u> (teeth eruption and fur development)	<u>Dams:</u> Decreased liquid intake. No effects on body weight, food consumption or pregnancy duration, litter size.  <u>Pups:</u> <u>Birth Weight:</u> both male and female pups of quinine groups were significantly lower than controls.  <u>Malformations:</u> <u>Quinine group:</u> 2 fetuses affected. One had syndactylia in the right forelimb; the other had anophthalmia involving the right eye.  <u>Postnatal Development:</u> comparing to controls significantly delayed eye opening and teeth eruption. No effects on surface righting, ears unfolding and opening, fur development.  Postnatal growth was reported as "impaired". Body weight data not given in the paper.  Saccharin included as a control group and had no effects other than on postnatal growth (where it was "impaired").

Table 5.5  
Ototoxicity of Quinine

Authors	Species/ Model	Dose(s)	Observations
Jasterboff et al., 1991 (as reviewed by Jung et al., 1993)	Rat/ Conditioned-suppression	200 mg/kg s.c.	Licking behavior changes suggestive of dose-dependent tinnitus; blocked by a Ca <sup>2+</sup> -channel blocker
Ruedi et al., 1952; Covell, 1938 (as reviewed by Jung et al., 1993)	Guinea pig	"high dose"	Loss of outer hair cells
Lee and Jung, submitted (as reviewed by Jung et al., 1993)	Guinea pig	5 µg/5 µL topical (to round window membrane)	No loss of hair cells at 2 hours when hearing loss maximal
Karlsson and Flock, 1990 (as reviewed by Jung et al., 1993)	Isolated guinea pig outer hair cells in tissue culture	0.78-7.8 µg/mL	Elongation followed by shortening
Smith et al., 1985 (as reviewed by Jung et al., 1993)	Guinea pig	200 mg/kg i.m.	Selective effect on compound action potential in response to high- and low-frequency bursts; less or intermediate. Cochlear microphonic suppressed
Lee et al., 1992	Chinchilla	150 mg/kg systemic and 5 µg/5 µL topically (to round window membrane)	Systemic dosing produced hearing loss of 20 dB with peak at 8 hrs and recovery by 18 hrs. Topical dosing produced hearing loss for 2 hrs with reversal by 9 hours and parallel initial decrease in cochlear blood flow followed by reversal.

**OVERALL CONCLUSIONS AND RECOMMENDATIONS**

Conclusions: The submission is acceptable with regards to pharmacology and toxicology issues.

Unresolved toxicology issues (if any): none

Recommendations: none

Suggested labeling:

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