Review Article

Animal Embryotoxicity Studies of Key Non-Artemisinin Antimalarials and Use in Women in the First Trimester

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The World Health Organization currently recommends quinine plus clindamycin for use against malaria in the first trimester. This may soon change to recommending artemisinin-based combination therapies (standard duration of dosing = 3 days). The non-artemisinin partner drugs include amodiaquine, lumefantrine, mefloquine, piperaquine, sulfadoxine-pyrimethamine, and pyronaridine. For quinine, clindamycin, and mefloquine and the combinations of sulfadoxine-pyrimethamine and artemether-lumefantrine, there are reports (including studies without internal comparison groups) that combined describe 304 to >1100 exposures of women in the first trimester for each drug with no conclusive evidence of adverse effects on pregnancy at therapeutic doses. This is despite the fact that all of these drugs or drug combinations caused embryo deaths and/or malformations in at least one animal species and all except lumefantrine had at least one exposure ratio <1. It now seems that these animal studies overestimated the risk of developmental toxicity in women with malaria. Three other non-artemisinins (amodiaquine, piperaquine, and pyronaridine) have few or no reported exposures in women in the first trimester and have exposure ratios ≤2 based on studies in pregnant rats and rabbits with dosing throughout organogenesis. However, none of these drugs caused embryo deaths or malformations in pregnant rats and rabbits with the exception of pyronaridine, which caused embryo deaths only at a dose that was excessively toxic to the mothers. Thus, for amodiaquine, piperaquine, and pyronaridine, the testing in animals did not reveal findings of concern and the exposure ratios were in the range of the other non-artemisinin antimalarials described above.

Birth Defects Research 109:1075–1126, 2017. © 2017 The Authors. Birth Defects Research Published by Wiley Periodicals, Inc.

Key words: animal studies; malaria; antimalarials; artemisinins; embryotoxicity; teratogenicity; ACTs

Introduction

Among the six species of malarial parasites, the most widespread and deadly is Plasmodium falciparum because the parasites generate proteins that move to the surface of the infected red blood cells (RBCs) and adhere the infected RBCs to the vascular endothelium, causing them to evade clearance in the spleen (Guerin et al., 2002). Adults can acquire some protective immunity. However, children and pregnant women are especially susceptible to severe malarial infections (Brabin, 1997; Granja et al., 1998; Steketee et al., 2001; Sachs and Malaney, 2002; Menendez, 2006). Pregnant women, particularly primagravidae, are sensitive because any acquired immunity does not prevent the binding of infected RBCs to the naive trophoblast. The resultant accumulation of infected RBCs in the placenta accounts for an increased parasitic load in these women which, in turn, is responsible for maternal morbidity and mortality and adverse effects on the conceptus, such as intrauterine death, stillbirth, preterm labor, low birth weight (Suh and Keystone, 1996; Brabin and Piper, 1997; Deen et al., 2001; Deen and von Seidlein, 2002; McGready et al., 2012) and vertical transmission of the disease (Bialek and Knobloch, 1999). Thus, treatment of malaria during pregnancy can be beneficial to the offspring as well as the mother.

In a technical report of the World Health Organization (WHO, 2001) and then in the first edition of the WHO Guidelines for the Treatment of Malaria (WHO, 2006), the recommendation of the WHO for falciparum malaria has been to administer combinations of independently effective antimalarials with different modes of action and, in particular, combinations including an artemisinin (referred to as artemisinin-based combination therapy, or ACT). The rationale was that a combination was often more effective and that, if a mutant parasite resistant to one of the antimalarials arose, it would be killed by the other antimalarial. The strategy was to yield mutual protection that would delay the development of resistance.

ACTs are particularly effective because artemisinins reduce parasite numbers by 10,000-fold in each asexual cycle compared with 100- to 1000-fold for other antimalarials. Artemisinins are eliminated rapidly and, when combined with a slowly eliminated antimalarial, the course of effective treatment is shortened from 7 days to 3 days. In the first edition of the Guidelines for the Treatment of Malaria (WHO, 2006), ACTs represent one option recommended for use in the second and third trimesters. In the third edition of the Guidelines for the Treatment of Malaria (WHO, 2015a), it is strongly recommended that one of the
following ACTs be used to treat all children and adults with uncomplicated *P. falciparum* malaria except for women in the first trimester (artemisinin listed on the left below):

- Artemether + lumefantrine
- Artesunate + amodiaquine
- Artesunate + mefloquine
- Dihydroartemisinin (DHA) + piperaquine
- Artesunate + sulfadoxine + pyrimethamine.

For women in the first trimester with uncomplicated malaria, 7 days of quinine + clindamycin is currently recommended or, in the absence of clindamycin, just quinine. For women in the first trimester with severe malaria, artemether administered intravenously or intramuscularly is recommended.

The WHO recommendation that artemisinins not be used in the first trimester for uncomplicated malaria was first published in 2003 based largely on feedback from committees of experts that reviewed unpublished reports of studies of artemisinins in pregnant animals (WHO, 2003). The embryo-fetal development (EFD) studies of artesunate in rats and rabbits were eventually published (Clark et al., 2004) and additional studies of artesunate showed that the target for artesunate in the embryo of the rat (White et al., 2006; Longo et al., 2006) and monkey (Clark et al., 2008a) was the circulating primitive erythroblast. It was also shown that the effects of artesunate, artemether, DHA and artemether in the rat were similar (cardiac and skeletal malformations and embryonic death) and, therefore, that the developmental toxicity of artemisinins in rats represented a class effect (Clark et al., 2008b). In general, these findings occurred at relatively low exposures in rats, rabbits, and monkeys (see Clark, 2009). Based on a critical period study in rats (White and Clark, 2008) and knowledge of when the primitive erythroblasts are predominant in the circulation of the human embryo, it has been estimated that the putative sensitive period in humans (counting from the first day of the last menstrual period) would be the beginning of gestational Week 6 to the end of gestational Week 12 (Clark, 2009).

In recent years, there has been an increasing number of reports on the use of artesunate and ACTs in the first trimester which have not revealed an effect on pregnancy (e.g., Adam et al., 2009; Manyando et al., 2010, 2015; Rulisa et al., 2012; Mosha et al., 2014; Dellicour et al., 2015, Dellicour et al., 2017; Tinto et al., 2015; Moore et al., 2016) leading the World Health Organization (WHO) to consider recommending ACTs for use in the first trimester. To aid in the evaluation of the safety of ACTs in the first trimester, this article will review the results of the pregnant animal studies of the non-artemisinin partner drugs in the ACTs mentioned above and then compare these animal results to what is known about the use of these drugs in women in the first trimester. Similar results are reported for quinine and clindamycin. The possible risk of adverse effects on pregnancy resulting from exposure in the second and third trimesters is not addressed in the review. Except where noted, drugs were administered orally in both the animal and clinical studies. To assist the reader in the interpretation of the findings in the animal studies, a discussion of factors involved in assessment of risk to pregnancy based on animal studies is provided in the following section.

### Developmental Toxicity Studies in Animals and Their Interpretation

Some basic information about embryology, teratology, and the conduct and interpretation of EFD toxicity studies in animals is presented here. Included is information on regulatory guidelines regarding study design and on key factors involved in the assessment of risk to human pregnancy based on the results of EFD studies in animals.

### Terminology

During in utero development, the period of the development of the major organs is referred to as organogenesis and extends from implantation up to the closure of the secondary palate. Before the closure of the secondary palate, the conceptus is referred to as an embryo and thereafter as a fetus. Effects on development can include malformations that are largely the result of a mistake in the formation of an organ during organogenesis and, therefore, are induced almost entirely during the embryonic period. In humans, implantation occurs on postconception Day 6 to 7 and the secondary palate closes between postconception Days 56 and 58 (Shepard, 2007) and thus most malformations are induced during the first two-thirds of the first trimester. There are examples of the conceptus being sensitive to other types of serious drug-induced injury throughout gestation.

Terms to be used in this article to describe adverse effects on the conceptus are as follows. Toxicity that occurs during prenatal and/or postnatal development is referred to as developmental toxicity and can consist of effects on growth, development (including functional and behavioral deficits), and/or survival. Developmental toxicity that is induced during the embryonic period is referred to as embryotoxicity and that during the fetal period as fetal toxicity. The terms teratogen and teratogenicity are reserved to refer to agents that cause malformations.

Regarding the use of the term adverse, it has become common to dismiss some findings in general toxicity studies as being nonadverse (e.g., Karbe et al., 2002; Williams and Latropoulos, 2002). However, some of the criteria for being considered nonadverse in a general toxicity study do not necessarily apply to developmental and reproductive...
toxicity studies. For example, when an effect of treatment is transient, that is considered to be a reason to dismiss that effect as being nonadverse in a general toxicity study but, in a pregnant animal, a transient effect on the mother could nevertheless cause or contribute to developmental toxicity. Also, embryo death and malformation are clearly adverse but otherwise there is no consensus on what constitutes adverse and nonadverse developmental effects. The practice of dismissing some effects as being nonadverse is not observed in this article, and no observed effect levels (NOELs) for maternal (mNOEL) and developmental (dNOEL) effects are identified (as opposed to no observed adverse effect levels, NOAELs).

REGULATORY GUIDELINES FOR TESTING NEW DRUGS FOR DEVELOPMENTAL TOXICITY IN ANIMALS
In 1966, triggered by the thalidomide tragedy, the US Food and Drug Administration (FDA) issued guidelines specifying developmental and reproductive toxicity studies to be conducted on prospective new drugs (FDA, 1966). Starting in 1978, the FDA required that these studies comply with Good Laboratory Practice (GLP) regulations (FDA, 1978). The 1966 FDA Guidelines were superseded in 1993 by guidelines from the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH, 1993). The ICH Guidelines for developmental toxicity studies were last updated in 2005 (ICH, 2005). The design of studies on the effects on embryo-fetal development (i.e., EFD studies, formerly known as Segment II or teratology studies) has remained essentially unchanged and includes treatment throughout organogenesis. The treatment group size indicated for rodent and rabbit studies in the current ICH Guideline is 16 to 20 pregnant females. The guidelines specify that there be some minimal maternal toxicity at the high dose.

REGULATORY GUIDANCE REGARDING ASSESSMENT OF RISK TO HUMAN PREGNANCY BASED ON DEVELOPMENTAL TOXICITY STUDIES IN ANIMALS
Both the Committee for Medicinal Products for Human Use (CHMP) of the European Medicines Agency (EMEA, 2008) and the FDA (2011) have issued guidance for assessing the level of concern for adverse effects on human pregnancy based on data from developmental and reproductive toxicity studies in animals. These documents list six or seven factors as being important when assessing risk to human pregnancy in the first trimester based on the results of these studies. According to the 2011 FDA Guidance, an analysis of these factors for each compound can be used to determine a summary risk conclusion which may include wording such as “does not appear to increase risk,” “may increase risk,” or “predicted to increase risk” (FDA, 2011). Some of the key factors to be considered when assessing risk to human pregnancy are elaborated below.

MATERNAL TOXICITY
As stated above, the ICH Guidelines specify that there be some minimal maternal toxicity at the high dose of EFD studies. It is common for maternal toxicity to be accompanied by developmental toxicity. For example, among 177 studies in rodents or rabbits in which there was maternal toxicity, 133 (75%) of them also had effects on embryo survival (Khera, 1985). Furthermore, maternal toxicity can be a confounding factor when interpreting developmental toxicity studies in animals because it can cause developmental effects (see Carney, 2010). Even common manifestations of maternal toxicity can impact development. For example, dietary deprivation during organogenesis in rabbits has been shown to cause resorptions and decreased fetal weight in rabbits (Matsuzawa et al., 1981; Clark et al., 1986) and deprivation of both food and water has been shown to cause cleft palate in mice (Tocco et al., 1987).

In some cases, it seems clear that developmental toxicity is secondary to maternal toxicity. For example, hemolytic anemia induced by the nonsteroidal anti-inflammatory drug diflunisal induced axial skeletal malformations in rabbits (Clark et al., 1984). In other cases, it is not clear that the developmental toxicity is secondary to the maternal toxicity but it seems likely that the maternal toxicity at least contributed to the developmental effects. For example, in a rabbit EFD study of the antimalarial pyronaridine (W. Yu, unpublished data; see the Pyronaridine section), the high dose of 120 mg/kg/day (N = 21) was excessively toxic with one maternal death, eight abortions, and prolonged anorexia with associated effects on maternal body weight gain. At that dose, there were increased resorptions plus dead fetuses compared with control.

KEY DEVELOPMENTAL ENDPOINTS
In humans, the type of developmental toxicity that has the greatest social impact and the greatest stigma is the induction of malformations (i.e., teratogenicity). Accordingly, there is emphasis on whether a drug causes malformations in animals and compounds are commonly classified into one of two categories: teratogenic or nonteratogenic. This practice is not very informative for three reasons. First, most small, biologically active compounds can be found to be teratogenic in animals. For example, Frankos (1985) reported that 102 (70%) of 146 compounds (mostly drugs) with no positive reports in humans were teratogenic in at least one animal species. Second, even though sometimes related to excessive maternal toxicity, embryo death can also be a reason for concern. Even if one is primarily concerned about malformations in humans, it is not uncommon for a test agent to cause embryo deaths in one species and malformations in another. Embryo deaths sometimes result from malformations and there could be species differences in the ability of the malformed embryo...
to recover and survive to term. Also, it is possible that embryo deaths could mask the potential to cause malformations that would be expressed at slightly lower doses and/or with a shorter treatment period. Third, the binary categorization does not take into account differences in dose or exposure at the toxic dose in animals compared with that at therapeutic regimens in humans.

The type of malformation induced is also important. Certain types of malformations occur commonly at maternally toxic doses (e.g., axial skeletal defects in rats and rabbits; Khera, 1985). Whether these malformations can be determined to be secondary to the maternal toxicity, they are considered to be of less concern than unusual malformations.

SELECTIVE DEVELOPMENTAL TOXICANTS
It is common to observe embryo deaths and malformations with drugs and candidate drugs in animals. It is uncommon for a test agent to induce these effects at nonmaternally toxic doses, that is, to be selective for the embryo. It is an unusual feature of thalidomide that it caused high incidences of unusual malformations at nonmaternally toxic doses in rabbits and primates (e.g., Christian et al., 2007). Artesunate, artemether, and DHA have also caused embryo deaths and/or malformations at nonmaternally toxic doses when tested in rats, rabbits, and/or monkeys (Clark et al., 2004, 2008a,b, 2016).

CRITICAL PERIODS
Malformations in a particular organ can only be induced by the disruption of embryogenesis during certain specific stages related to the development of that organ (Wilson, 1977). This means that a teratogen can only induce a certain type of malformation when administered on specific gestational days (GDS). For rodents and rabbits, for which the duration of organogenesis is approximately 9 to 13 days, the sensitive period for the induction of a particular malformation is generally 1 to a few days long. Not much information is available regarding critical periods in humans for which the duration of organogenesis is approximately 49 to 52 days. One example is provided by thalidomide for which the critical period for the induction of the characteristic malformation phocomelia was postconception Days 38 to 49 (Schardein and Macina, 2007).

One impact of this phenomenon is that the potential for a given drug to cause a certain teratogenic effect can only be ascertained following the administration of the drug on those specific gestational days. If the treatment period in a clinical study was short and the exposures of pregnant women were scattered throughout the first trimester (or, more commonly, primarily late in the first trimester), then it should be recognized that only a portion of these exposures would have occurred during the sensitive period. In most cases, the sensitive period would fall sometime within the period of organogenesis (approximately GD 6/7 to 56/58).

DURATION OF DOSING COMPARED WITH THE THERAPEUTIC DOSING REGIMEN
Depending on the purpose of the study, the duration of dosing in an EFD study can range from a single day to daily dosing throughout organogenesis. Studies conducted to support the regulatory registration of drugs typically comply with regulatory guidelines which specify treatment throughout the period of organogenesis. As mentioned above, the duration of organogenesis in rodents and rabbits is much shorter (approximately 9 to 13 days) than it is in humans (approximately 49 to 52 days). Therapeutic regimens for ACTs typically involve treatment for 3 days and, for quinine, 7 days, a fraction of the period of organogenesis.

In general, longer treatment periods in pregnant animal studies lead to greater maternal toxicity, which then limits the doses that can be tested. In some cases, dosing for longer periods at maternally toxic doses enhances developmental toxicity (e.g., lersivirine in rabbits; Campion et al., 2012). In other cases, though, dosing throughout organogenesis can hide developmental toxicity. For example, when diflunisal was administered throughout organogenesis at a near-lethal dose of 100 mg/kg/day, there was mean maternal body weight loss but no embryo deaths or malformations (Nakatsuka and Fujii, 1979). However, when administered at 250 mg/kg/day just on GD 9 and 10, there was a treatment-related increase in the incidence of ventricular septal defects (Cappon et al., 2003). Ibuprofen provides a similar example (Adams et al., 1969; Cappon et al., 2003). Thus, having an extended dosing period where the dose is limited by maternal tolerance can prevent reaching dose levels that would cause developmental toxicity during shorter, sensitive periods. Developmental toxicity can also be enhanced by longer treatment periods when doses are not limited by maternal tolerance (e.g., artesunate in monkeys, Clark et al., 2008b; also, see Davis et al., 2009). Thus, in the situation where the therapeutic dosing regimen is short, these effects of dosing throughout organogenesis can confound the assessment of risk to pregnancy in humans.

EXPOSURE RATIOS AND HUMAN EQUIVALENT DOSE
Animal to human exposure ratios are commonly evaluated when assessing the risk of a compound to human pregnancy. A typical approach is to calculate the ratio of a kinetic metric such as the area under the curve (AUC) or maximum concentration (Cmax) at the highest dNOEL in the animal species to that at the therapeutic dose in humans. These ratios will be referred to as the AUC and Cmax ratios. It should be noted that the degree of confidence that a particular dose is truly a NOEL depends on the group size, and there is much more confidence when
| Species (strain - if specified) | Route/treatment period | Doses | Group size (litters) | dNOEL/dLOEL | HED (mg/kg) | Maternal toxicity | Developmental findings | Reference |
|-------------------------------|------------------------|-------|---------------------|-------------|-------------|-----------------|---------------------|-----------|
| Rat (Wistar and other strains) | i.m., i.p., p.o., s.c. on GD 7 to 14 | 50 mg/kg/day | Not provided | dNOEL = 50 mg/kg/day | 8.2 mg/kg/day | - | One litter (route not specified) had a multiply malformed fetus and 2 dead fetuses | Neuweiler and Richter, 1964 |
| Rat (Sprague-Dawley) | Free access to 0.25 mg/ml in drinking water from 2 weeks before mating through lactation | Up to 150 mg/kg b.i.d | Not provided | dNOEL = 150 mg/kg/day | 24.6 mg/kg/day | - | No developmental effects | Savini et al., 1971 |
| Rat (Sprague-Dawley) | p.o. on GD 7 to 18 | 50, 100 and 200 mg/kg/day | 25 | dNOEL = 100 mg/kg/day | 16 mg/kg/day | Decreased body weight gain and increased water consumption at 100 and 200 mg/kg/day | At 200 mg/kg/day, 6% decrease in fetal weight, delayed ossification and increased visceral and skeletal anomalies but no malformations | Colley et al., 1989 |
| Rabbit | p.o. during unspecified 10-day periods of gestation | ~6.5, 19 and 32 mg/day | 1 litter of 2 to 6 kits | dEL = 6.5 mg/day (≤ 1.6 to 3 mg/kg/day) | ~0.5 to 1 mg/kg/day | - | ~6.5 mg/day: degeneration in the auditory nerve, spinal ganglion, and peripheral neuron at 2 months of age | West and Wichita, 1938 |
the group size is 20 than when it is 8 or less. Also, exposure ratios are not precise. The dNOEL can vary from study to study and is dependent upon the selection of doses and, in particular, how closely the doses are spaced. Also, the AUC and C<sub>max</sub> at the dNOEL can vary depending on the day of gestation selected for their measurement.

The kinetic metric most closely correlated with developmental toxicity (i.e., C<sub>max</sub> AUC, or other) varies among compounds (see Wier, 2011) and is not known for a specific compound unless carefully studied. The AUC ratio is used more commonly, is sometimes available when C<sub>max</sub> is not, and will be referred to predominantly in this review.

For many drugs, there are no published kinetic data at appropriate doses for the relevant animal species and/or humans. In these cases and sometimes even when appropriate kinetic data are available, animal to human comparisons are made using allometric scaling based on dose per body surface area, that is, mg/m<sup>2</sup>, which adjusts for species differences in physiological parameters (Freireich et al., 1966; Schein et al., 1970; EPA, 1992; Lowe and Davis, 1998; Reagan-Shaw et al., 2008). This is done by first converting the animal dose at the no effect level to the human equivalent dose (HED) by dividing the animal dose by a species-dependent factor (e.g., 12.3 for mice, 6.2 for rats, and 3.1 for rabbits and cynomolgus and rhesus monkeys). The animal HED at the dNOEL is then compared with the human therapeutic dose. This ratio will be referred to here as the HED ratio. The FDA states that HEDs should be used when making animal to human extrapolations for the purpose of selecting starting doses in clinical trials in healthy volunteers (FDA, 2005). HEDs have also been used for cross-species comparisons of dNOELs (Theunissen et al., 2016) and doses have been expressed as mg/m<sup>2</sup> when describing results of reproductive toxicity studies in product labels (e.g., Prozac, Eli Lilly & Co. 1987; clindamycin, Pharmacia and Upjohn, 2008).

The AUC, C<sub>max</sub> and HED ratios are to be collectively referred to as exposure ratios. There is no agreed upon standard for what is an acceptable exposure ratio for developmental toxicity in animals although the FDA (2011) has characterized an exposure ratio < 10 as being low and a cause for increased concern and one > 25 as being high and a cause for decreased concern. However, these statements about concern are not based on empirical evidence and cannot be translated into a probability that a given drug will cause developmental toxicity when administered at therapeutic doses to women in the first trimester.

**Quinine**

**STUDIES IN PREGNANT ANIMALS**

Quinine has been tested for developmental toxicity in a variety of animal species. The results are summarized in Tables 1 and 2.
| Species      | Route/ treatment period | Doses               | Group size (litters) | dNOEL/ dLOEL          | HED                  | Maternal toxicity | Developmental findings                                                                 |
|--------------|-------------------------|---------------------|----------------------|-----------------------|---------------------|--------------------|--------------------------------------------------------------------------------------------|
| Chinchilla   | 4-8 days during the first half of pregnancy | 90 to 150 mg/kg/day | -                    | dLOEL = 130 mg/kg/day | 42 mg/kg/day        | -                  | 130 mg/kg/day: 35% resorptions; among newborns, 3.2% had brain deformities (anencephaly, microcephaly), 13% were born dead, and 50% had underdeveloped brains | Belikina, 1958 |
| Chinchilla   | s.c., 6 to 8 days during GD 4 to 14 (day after mating = GD 1) | 90 to 100 mg/kg/day | 3 (9 live fetuses)   | dEL = 90 to 100 mg/kg/day | ~31 mg/kg/day       | 3 deaths among 74 quinine-treated females | All 9 fetuses were small and *enfeebled* with small brains | Klosovskii, 1963 |
| Chinchilla   | s.c., 1 to 8 days during GD 4 to 14 | 130 to 150 mg/kg/day | 28 (107 live fetuses) | dEL = 130 to 150 mg/kg/day | ~45 mg/kg/day       | 43% dead fetuses compared to 4% in control; 3 fetuses from 1 litter had head malformations (anencephaly, microcephaly, hydrocephaly); 39 of 107 fetuses were *enfeebled* with small brains | |
| Mouse        | p.o. on GD 6, 10, 6 to 10, or 0 to 17 | 125, 250, and 500 mg/kg/day | -                    | dEL = 250 mg/kg/day | 20 mg/kg/day        | -                  | Embryolethality and growth retardation but no malformations “at higher doses” | Tanimura and Lee, 1972 |
| Guinea Pig   | s.c. during unspecified 3-week period of gestation | 200 mg/kg/day | -                    | dEL = 200 mg/kg/day | 43 mg/kg/day        | -                  | Fetus(es?) had alterations in the mitochondria within the stria vascularis of the cochlear duct | Covell, 1936 |
| Species | Route/treatment period | Doses | Group size (litters) | dNOEL/dLOEL | HED | Maternal toxicity | Developmental findings | Reference |
|---------|------------------------|-------|---------------------|-------------|-----|------------------|-----------------------|-----------|
| Guinea Pig | p.o. (? during unspecified varying periods of gestation | Various | 1 | dEL = 42 mg/day for 115 days \(\approx\) 40 mg/kg/day | 9 mg/kg/day | - | Hemorrhages within the fetal cochlea | Mosher, 1938 |
| Dog | i.m. GD 18 to 48 | 15, 30 and 50 mg/kg/day | 4 | dEL = 15 mg/kg/day | 8 mg/kg/day | In adult dogs of unspecified gender, the administration of 75 mg/kg/day for 12 days was not lethal but the administration of 100 mg/kg/day caused death within 5 days for 4 of 6 adult dogs | 43.2% resorptions at 15 mg/kg/day compared to none in controls; the dose killing the embryos was 16-22 times less than the toxic dose for the adult dog; no macroscopic anomalies of the fetus were observed | Savini et al., 1971 |
| Two species of macaques | p.o. on GD 27 | 1 M. f. monkey treated at 20 mg/kg/day; M. m. monkeys: 3 at 20 mg/kg/day, 1 at 100 mg/kg/day and 1 at 200 mg/kg/day | 7, 32, and 65 mg/kg/day | dNOEL = 20, 100 and 100 mg/kg/day | Retroplacental hemorrhages in single M. m. monkeys at 20 and 100 mg/kg/day (c-sectioned on GD 60 and 62, respectively) were interpreted to demonstrate potential abortive effects | No malformations in 6 monkey fetuses | Tanimura, 1972 |

*Body weight was 2.5 to 4.1 kg and the factor used to calculate the HED was the same as that for rabbits (i.e., divide dose by 3.1).*

GD, gestational day; dEL, developmental effect level dNOEL, developmental no effect level; dLOEL, developmental low effect level; HED, human equivalent dose; i.m., intramuscular; p.o., oral; s.c., subcutaneous.
Brain and/or inner ear abnormalities were seen in rabbits at \(\sim\)1.6 to 3 mg/kg/day orally (p.o.) (HEDs = 0.5 to 1 mg/kg/day; West and Wichita, 1938), chinchillas at 130 mg/kg/day (HED = 42 mg/kg/day; Belkina, 1958) and 90 to 150 mg/kg/day (HED = 31 to 45 mg/kg/day; Klosovski, 1963), and in guinea pigs at \(\sim\)40 mg/kg/day (HED \(\geq\) 9 mg/kg/day; Mosher, 1938) and 200 mg/kg/day (HED = 43 mg/kg/day; Covell, 1936). Quinine-induced effects on embryo survival were observed in rabbits at 100 mg/kg/day (HED = 32 mg/kg/day; Savini et al., 1971), in chinchillas at 130 mg/kg/day (HED = 42 mg/kg/day; Belkina, 1958) and 130 to 150 mg/kg/day (HED \(\geq\) 45 mg/kg/day; Klosovski, 1963), in the mouse at 250 mg/kg/day (HED = 20 mg/kg/day; Tanimura and Lee, 1972), and in the dog at 15 mg/kg/day (HED = 8 mg/kg/day; Savini et al., 1971). The observation of retroplacental hematomas in single Macaca mulatta monkeys at 20 and 100 mg/kg/day (HED = 7 and 32 mg/kg/day, respectively) when cesarean-sectioned on GD 60 and 62, respectively, were interpreted to demonstrate potential abortive effects (Tanimura, 1972). In a non-GLP study in rats with a large group size (25) and external, visceral, and skeletal examinations of fetuses, there were no developmental effects at a maternally toxic dose of 100 mg/kg/day (HED = 16 mg/kg/day) and only minor developmental effects at 200 mg/kg/day (HED = 33 mg/kg/day; Colley et al., 1989).

With the exception of this last rat study, details about maternal toxicity in these studies were lacking. In the rabbit study with decreased embryo survival at 100 mg/kg/day (HED = 32 mg/kg/day; Savini et al., 1971), there was also 25% maternal mortality. In dogs, there appeared to be some selectivity of the developmental effect because there were apparently no maternal deaths at 15 mg/kg/day (HED = 8 mg/kg/day) which caused 43% embryonic resorptions and there were no deaths among an unspecified number of adults which all survived 12 days of treatment at 75 mg/kg/day (HED = 42 mg/kg/day; Savini et al., 1971).

**THERAPEUTIC DOSING REGIMEN**

A popular quinine treatment regimen is 10 mg salt/kg three times daily for 7 days (e.g., JHPIEGO, 2008). For a 50-kg person, that corresponds to 1.5 g/day. This regimen was used for most of the clinical studies discussed below. There has been mention of using higher doses to combat declining sensitivity in some areas (McGready et al., 1998). The WHO currently recommends the use of quinine in the first trimester with a therapeutic dosing regimen of 10 mg/kg salt (8.3 mg/kg base) twice daily for 7 days (i.e., 20 mg/kg/day; WHO, 2015a). For a 50-kg person, that corresponds to 1.0 g/day.

**EXPERIENCE IN PREGNANT WOMEN IN THE FIRST TRIMESTER**

In 7 studies involving >893 pregnant women who were treated with quinine for malaria during the first trimester, there were no clear effects of quinine on the rate of miscarriage or malformations compared with other antimalarial drugs (McGready et al., 1998, 2002, 2012; Nosten et al., 1999; Poespuprodjo et al., 2014; Dellicour et al., 2015; Moore et al., 2016). There were also no identifiable effects among women treated with quinine in the first 3 or 4 months of pregnancy in studies with no formal comparison group. Among these women there were 26 being treated for malaria (Adam et al., 2004a) and 139 women included in databases for which the reason for the quinine treatment is not given (Heinonen et al., 1977; Rosa, 1993, as reported in Briggs, 2015b).

In a meta-analysis of five studies including data from Moore et al., 2016, the risk of miscarriage was higher with first trimester quinine (incidence = 96/945 = 10.2%) compared with all artemisins and ACTs combined (incidence = 37/671 = 5.5%), but the hazard ratio for the two treatments (ratio of instantaneous risk calculated using the Cox proportional hazards model) was not statistically significant (Dellicour et al., 2017). The incidences of stillbirth for the two treatments were similar (11/615 = 1.8% for quinine versus 10/654 = 1.5% for artemisinins). Among 741 evaluated infants in the quinine group, there were 10 major congenital anomalies that occurred in eight infants (1.1%) which was similar to the incidence in the women without malaria and not treated with an antimalarial. Only one of these anomalies was of the central nervous system.

In another study, 70 pregnant women were treated in the first trimester with quinine only and 8 additional pregnant women with quinine plus artemether-lumefantrine (Mosha et al., 2014, which was included in Dellicour et al., 2017). Among those treated with quinine only, there were 3 miscarriages and 5 stillbirths. Among those treated with quinine plus artemether-lumefantrine, there were 2 miscarriages and no stillbirths. The incidence of miscarriages plus stillbirths in women treated with quinine, alone or combined with artemether-lumefantrine (10/78 = 12.8%), was statistically significantly greater (adjusted odds ratio [OR] = 2.5, 95% confidence interval [CI] = 1.3–5.1, \(p = 0.009\)) than the incidence for all other groups combined (96/1705 = 5.6%). Artemether-lumefantrine without quinine (13/172 = 7.6%) was not significantly different (adjusted odds ratio = 1.4, CI = 0.8–2.5, \(p = 0.295\)) from all other groups combined (93/1611 = 5.8%). There was only a single congenital anomaly in the quinine group. Thus, quinine exposure in the first trimester was associated with an increased risk of miscarriage/stillbirth compared with all other groups combined.

Malaria itself can cause miscarriage in the first trimester. In a study at the Shoklo Malaria Research Unit in Mae Sot, Tak, Thailand (McGready et al., 2012), 19% (3,198/16,668) of women with no malaria in the first trimester miscarried compared with 33% (163/496) of women with a single untreated episode of *P. falciparum* malaria in the
first trimester and 36% (150/412) of women with a single untreated episode of *P. vivax* malaria in the first trimester. The miscarriage rate was slightly lower in the malaria group treated with quinine in the first trimester (95/355 = 27%).

Quinine has been used with the intent to cause contraception (Klosovskii, 1963; Smit and McFadyen, 1998) and abortions (Klosovskii, 1963; Nishimura and Tanimura, 1976; Dannenberg et al., 1983; Smith, 1998) and to induce labor (Maxwell, 1908; Taylor, 1933; Robinson et al., 1963). There have been at least 94 case reports of the use of quinine in pregnancy (often in a failed attempt at abortion) being associated with congenital anomalies, particularly of the inner ear, eyes, and brain (e.g., hydrocephaly; Nishimura and Tanimura, 1976; Dannenberg et al., 1983; Sardein and Macina, 2007). Treatment was for malaria in only a few of these cases. At least 37 of these case reports, including 12 with partial or total hearing loss in the offspring, were associated with the use of quinine in the first trimester (Roberts, 1870; Dannenberg et al., 1983) and, in 35 of the cases, quinine was administered as a single dose in the range of 1 to 6 g.

**ASSESSMENT**

Quinine caused embryonic deaths in the rabbit, mouse, chinchilla, and dog at low effect levels for which the ratios of the corresponding HEDs to the therapeutic dose (20 mg/kg/day) ranged from 0.4 to 1.6. In a definitive study in the rat (Colley et al., 1989), the dNOEL was 100 mg/kg/day (HED = 16 mg/kg/day, HED ratio = 0.8). Also, there was evidence of specificity of quinine to affect the development of the brain and inner ear in the rabbit, chinchilla, and guinea pig.

Several substantial clinical studies of pregnant women with malaria treated in the first trimester with quinine have not revealed any quinine-induced increase in the incidences of congenital anomalies. The study of McGready et al. (2012), suggests that quinine may have a beneficial effect on the rate of miscarriage in pregnant women with malaria in the first trimester. The studies that show a higher rate of miscarriage in pregnant women with malaria treated with quinine compared with other antimalarial treatments may not indicate that quinine is causing miscarriages but rather that quinine is less effective at preventing malaria-induced miscarriages than other antimalarials. Thus, the effectiveness of a drug in preventing miscarriages in pregnant women with malaria may depend upon its effectiveness as an antimalarial.

**Therapeutic Dosing Regimen**

The oral dose of clindamycin to treat malaria is 10 mg/kg twice daily for 7 days (i.e., 20 mg/kg/day), and it is used in combination with quinine (WHO, 2015a).

**Experience in Pregnant Women in the First Trimester**

Sixteen women in Taiwan with pregnancies at a gestational age of 5 to 8 weeks and a previous spontaneous abortion were treated for threatened abortion (as indicated by severe abdominal pain) with 500 mg amoxicillin and 300 mg clindamycin 3 times daily for 7 days received (Ou et al., 2001). Fifteen or 16 of the women had term deliveries and no identified neonatal anomalies.
According to Briggs and Freeman (2015a): In a surveillance study of Michigan Medicaid recipients involving 229,101 completed pregnancies conducted between 1985 and 1992, 647 newborns had been exposed to clindamycin during the first trimester (includes both maternal systemic and nonsystemic administration) (F. Rosa, unpublished data, FDA, 1993). A total of 31 (4.8%) major birth defects were observed (28 expected). Specific data were available for six defect categories including (observed/expected) 5/6 cardiovascular defects, 0/1 oral clefts, 1/0.5 spina bifida, 1/2 polydactyly, 0/1 limb reduction defects, and 3/2 hypospadias. These data do not support an association between the drug and congenital defects.

ASSESSMENT

No developmental toxicity was observed in studies in pregnant rats and mice (HED ratios \(= 1.5\) and 0.7, respectively). Clindamycin is considered safe for use in pregnancy by WHO (2015a).

Amodiaquine

STUDIES IN PREGNANT ANIMALS

A standard package of ICH- and GLP-compliant developmental toxicity studies of amodiaquine and combinations of amodiaquine together with artesunate was conducted under the sponsorship of Sanofi-Aventis - EFD studies in rats (Tables 4 and 5) and rabbits (Tables 6 and 7) and a pre- and postnatal development (PPN) study in rats (Table 8).

**Rat EFD study.** Oral doses for the standard EFD study in Sprague-Dawley rats (R. Davies, unpublished data; treatment on GD 6 through 17) were based on the results of a dose-range finding (DRF) study in pregnant rats (W. Gaoua-Chapelle, unpublished data) in which adequate decreases in food consumption and maternal body weight gain were observed at oral doses of 30 mg/kg/day amodiaquine and the combination of 30 mg/kg/day amodiaquine plus 12 mg/kg/day artesunate (Table 4). Two groups receiving these doses were used in the definitive EFD study as well as a group receiving 12 mg/kg/day artesunate and combination groups receiving doses of amodiaquine plus artesunate of 3 and 1 mg/kg/day, respectively, and 10 and 4 mg/kg/day, respectively (Table 4).

The group treated with 30 mg/kg/day amodiaquine alone had decreases in maternal food consumption and body weight gain as well as a 6.5% decrease in fetal weight and delayed ossification. In the group treated with 12 mg/kg/day artesunate alone, there was a marked effect on embryo survival with 14 of 22 pregnant females having total litter loss as has been seen previously with artesunate (Clark et al., 2004). The group receiving both of these treatments (30 mg/kg/day amodiaquine plus 12 mg/kg/day artesunate) had less of an effect on embryo survival (5 of 19 litters totally resorbed) compared with the 12 mg/kg/day artesunate alone group. Also seen in that group was a 9.5% decrease in mean fetal weight and delayed ossification. There were no maternal or developmental effects in the group receiving 10 mg/kg/day amodiaquine plus 4 mg/kg/day artesunate.

Thus, when administered alone, 30 mg/kg/day amodiaquine (HED = 5 mg/kg/day) caused only minor developmental effects in rats and 10 mg/kg/day (HED = 1.6 mg/kg/day) amodiaquine coadministered with 4 mg/kg/day artesunate caused no developmental effects (i.e., the dNOEL).

Desethylamodiaquine and DHA are the primary metabolites of amodiaquine and artesunate, respectively. Both metabolites are active as antimalarials. Plasma concentrations of amodiaquine, desethylamodiaquine (desethylAQ), artesunate, and DHA were measured on GD 6 and 17 in the rat EFD study. Toxicokinetic data are shown in Table 5.

**Rat PPN study.** Two DRF studies (R. Davies, unpublished data; W. Gaoua-Chapelle, unpublished data) preceded the definitive rat PPN study (R. Davies, unpublished data) (Table 8). In all three DRF studies, the treatment period was GD 6 through postpartum day (PPD) 21 and the day...
| Study type | Group number | No. pregnant | Dose (mg/kg/day) | Maternal findings | Developmental findings |
|------------|--------------|--------------|------------------|------------------|-----------------------|
|            |              |              | Amodiaquine      | Artesunate       |                       |
| DRF        | 2            | 5            | 3                | -                | -                     |
|            | 3            | 6            | 10               | -                | -                     |
|            | 4            | 5            | 30               | -                | 27% decrease in body weight gain from GD 6-12; decreased food consumption |
|            |              |              |                  |                  | None noted            |
|            | 5            | 6            | 3                | 1                | -                     |
|            | 6            | 6            | 10               | 4                | -                     |
|            | 7            | 5            | 30               | 12               | 22% decrease in body weight gain from GD 6-12; decreased food consumption |
|            |              |              |                  |                  | None noted            |
| Definitive EFD | 2         | 22           | 3                | 1                | mNOEL                 |
|            | 3            | 21           | 10               | 4                | dNOEL                 |
|            | 4            | 19           | 30               | 12               | Increased resorptions including 5 of 19 pregnant females with total resorption, and postimplantation loss of 14% in the 14 litters with live fetuses; 9.5% decrease in fetal weight, delayed ossification |
|            | 5            | 20           | 30               | -                | 75% decrease in body weight gain from GD 6-9 and 14% decrease from GD 6 to 18; 8 to 9% decreases in food consumption from GD 6-15 |
|            | 6            | 22           | -                | 12               | mNOEL                 |

GD, gestational day; DRF, dose-range-finding study; EFD, embryo-fetal development study; mNOEL, maternal no effect level; dNOEL, developmental no effect level.
TABLE 5. Summary of Toxicokinetic Results in Pregnant Sprague Dawley Rats Administered Amodiaquine Alone or in Combination with Artesunate

| Group number | AQ (mg/kg/day) | AS (mg/kg/day) | GD (mg/kg/day) | Analyte | Amodiaquine | DesethylAQ | Artesunate | DHA |
|-------------|----------------|----------------|---------------|---------|-------------|------------|------------|-----|
|             | C$_{\text{max}}$ (ng/ml) | T$_{\text{max}}$ (h) | AUC$_{0-24\text{h}}$ (ng.h/ml) | C$_{\text{max}}$ (ng/ml) | T$_{\text{max}}$ (h) | AUC$_{0-24\text{h}}$ (ng.h/ml) | C$_{\text{max}}$ (ng/ml) | T$_{\text{max}}$ (h) | AUC$_{0-24\text{h}}$ (ng.h/ml) |
| 2           | 3              | 1              | 6              | BLQ     | NC           | NC         | 0.6        | 0.25 | NC  | 10.5        | 0.25    | 54.8 |
| 3           | 10             | 4              | 6              | BLQ     | NC           | NC         | 15.8       | 4.0  | 336 | 11.1        | 0.25    | 54.8 |
| 4           | 30             | 12             | 6              | 17.9    | 0.5          | 126        | 33.7       | 4.0  | 599 | 9.5         | 0.25    | 223 |
| 5           | 30             | -              | 6              | 18.8    | 4.0          | 266        | 178        | 4.0  | 3440| 5.5         | 0.25    | 128 |
| 6           | -              | 12             | 6              | 16.8    | 2.0          | 64.5       | 36.5       | 2.0  | 467 | -           | -       | -   |
|             | 17             | -              | -              | 54.4    | 4.0          | 609        | 235        | 24.0 | 5116| -           | -       | -   |

Three animals per group were sampled for each time-point and each animal was sampled three times on each day.

GD, gestational day; AQ, amodiaquine; AS, artesunate; BLQ, below the lower limit of quantitation (10 ng/ml); NC, not calculable due to low concentrations.
| Dose (mg/kg/day) | Study type | Amodiaquine | Artesunate | No. pregnant | Maternal findings | Developmental findings |
|-----------------|------------|-------------|-------------|---------------|-------------------|-------------------|
| 3               | DRF        | -           | -           | 5             | -                 | -                 |
| 10              | 3          | -           | -           | 6             | -                 | -                 |
| 30              | 3 1.2 6   | -           | -           | 71g loss in mean body weight from GD 6 to 9 compared to 22g gain in the control; decrease in food consumption during the dosing period diminishing from 26% between GD 6 and 7 to 13% between GD 15 and 19 | -                 |
| 3               | 10         | 4           | 4           | One maternal death not attributed to treatment | One female with total litter loss |
| 20              | 20         | 8           | 6           | -             | -                 |
| EFD 3 1.2 20    | 10         | 4           | 21 mNOEL   | dNOEL         | Increased mean postimplantation loss (33.9%) compared to control (6.0%) – only 37.2% of surviving fetuses were males; |
| 22.5            | 22.5       | 9           | 20 mNOEL   | dNOEL         | Increased mean postimplantation loss (57.1%) compared to control (6.0%) – only 17.1% of surviving fetuses were males; decrease in fetal weight compared to control; 3 fetuses from 2 litters had multiple CV malformations including right-sided aortic arch and absent innominate artery, these 3 fetuses plus 6 others from a total of 5 litters had CV variations – absent brachiocephalic trunk and/or short innominate artery | |
| -               | - 9 20     | 68g loss in mean body weight from GD 6 to 9 compared to 30g gain in the control; 28 to 43% decreases in mean food consumption during the dosing period | dNOEL |
| -               | 11 females aborted between GD 20 and 25; mean body weight remained the same on GD 15 and 19 compared to a 63g gain in the control group during this period, likely largely related to the death of conceptuses | Increased mean postimplantation loss (57.1%) compared to control (6.0%) – only 17.1% of surviving fetuses were males; decrease in fetal weight compared to control; 3 fetuses from 2 litters had multiple CV malformations including right-sided aortic arch and absent innominate artery, these 3 fetuses plus 6 others from a total of 5 litters had CV variations – absent brachiocephalic trunk and/or short innominate artery |

CV, cardiovascular; GD, gestational day; DRF, dose-range-finding study; EFD, embryo-fetal development study.

mNOEL = maternal no effect level; dNOEL = developmental no effect level.
when the delivery of a litter was complete was defined as PPD 1. The DRF studies established that the dose levels of 36 mg/kg/day amodiaquine alone, 14.4 mg/kg/day artesunate alone, or the combination of 30 mg/kg/day amodiaquine plus 12 mg/kg/day artesunate caused excessive toxicity and were unsuitable for use in the definitive PPN study which had three treatment groups consisting of combinations of amodiaquine + artesunate (3 + 1, 10 + 4, and 20 + 8 mg/kg/day, respectively) as well as groups treated with 20 mg/kg/day amodiaquine alone and 8 mg/kg/day artesunate alone (Table 8).

In the 20 mg/kg/day amodiaquine alone group of the PPN study, there were decreases in maternal body weight gain during gestation (45% between GD 6 and 9 and 12% between GD 6 and 20) and decreases in food consumption during gestation and the first 2 weeks postpartum (Table 8). Mean pup weights in this group were 6 to 9% lower than control on PPD 1 through 21. There was no effect on the survival of the offspring. In the 8 mg/kg/day artesunate alone group, only 6 of 27 pregnant females delivered live litters and one of these died within the first 24 hr after delivery. There was a 74% decrease in mean body weight gain between GD and GD 20 in this group which was largely related to the death of conceptuses.

When 20 mg/kg/day amodiaquine was coadministered with 8 mg/kg/day artesunate, there was no effect on the number of pregnant females delivering live offspring (19 of 23 pregnant females delivered live offspring compared with 25 of 28 in the control) although 11 pups from three litters did not survive the first 24 hr after delivery (compared with none in the control). Similar findings were observed in the 10 mg/kg/day amodiaquine + 4 mg/kg/day artesunate group in which all 25 pregnant females delivered live litters and 13 pups from three litters (9 from one litter) died within the first 24 hr after delivery. Also in the 20 + 8 mg/kg/day and 10 + 4 mg/kg/day combination groups, there were effects on maternal body weight gain and food consumption as shown in Table 8 and, in the 20 + 8 mg/kg/day group, mean pup weights were 8 to 12% lower than control on PPD 1 through 21. There were no observed effects of treatment in any group on physical development, the age of preputial separation or vaginal opening, the acoustic startle test, pupillary constriction, the water-maze test, or locomotor activity.

Thus, when administered alone, 20 mg/kg/day amodiaquine (HED = 3 mg/kg/day) caused only minor effects in rat pups and 3 mg/kg/day (HED = 0.5 mg/kg/day) amodiaquine coadministered with 1 mg/kg/day artesunate caused no developmental effects (i.e., the dNOEL).

### Rabbit EFD study

Oral doses for the standard EFD study in New Zealand White rabbits (R. Davies, unpublished data; treatment on GD 6 through 19) were based on the results of a DRF study in pregnant rabbits (R. Davies, unpublished data) in which the dose of 30 mg/kg/day amodiaquine caused 13 to 26% decreases in mean food consumption during the treatment period and a mean loss in body weight of 72 g between GD 6 and 9 compared with a 23 g gain in the control. In the definitive EFD study, groups were treated with 22.5 mg/kg/day amodiaquine alone, 9

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**TABLE 7. Summary of Toxicokinetic Results in Pregnant New Zealand White Rabbits Administered Amodiaquine Alone and in Combination with Artesunate**

| Group number | Dose (mg/kg/day) | Amodiaquine | DesethylAQ | Amodiaquine | DesethylAQ | Amodiaquine | DesethylAQ | Amodiaquine | DesethylAQ | Amodiaquine | DesethylAQ | Amodiaquine | DesethylAQ |
|--------------|-----------------|-------------|------------|-------------|------------|-------------|------------|-------------|------------|-------------|------------|-------------|------------|
|              | C<sub>max</sub> (ng/ml) | T<sub>max</sub> (h) | AUC<sub>0-24h</sub> (ng.h/ml) | C<sub>max</sub> (ng/ml) | T<sub>max</sub> (h) | AUC<sub>0-24h</sub> (ng.h/ml) | C<sub>max</sub> (ng/ml) | T<sub>max</sub> (h) | AUC<sub>0-24h</sub> (ng.h/ml) | C<sub>max</sub> (ng/ml) | T<sub>max</sub> (h) | AUC<sub>0-24h</sub> (ng.h/ml) |
| 2            | 3               | 1.2         | 6          | 34.8        | 4          | 326         | 79.1        | 4          | 1264       | 0.99        | 0.25        | NC          | BLQ         | NA          |
| 3            | 10              | 4           | 6          | 138         | 2          | 1187        | 222         | 4          | 3537       | 2.27        | 0.25        | NC          | 86.4        | 0.25        | 77.8        |
| 4            | 22.5            | 9           | 6          | 242         | 2          | 2098        | 468         | 4          | 6603       | 4.56        | 0.25        | NC          | 120         | 0.25        | 123         |
| 5            | 22.5            | -           | 6          | 238         | 2          | 2117        | 457         | 2          | 5352       | -           | -           | -           | -           | -           |
| 6            | -               | 9           | 6          | -           | -          | -           | -           | -          | -          | -           | -           | -           | -           |
| 18           | -               | -           | -          | -           | -          | -           | -           | -          | -          | -           | -           | -           | -           |

Three animals per group were sampled for each time point, and each animal was sampled 3 times on each day.

GD, gestational day; AQ, amodiaquine; AS, artesunate; BLQ, below the lower limit of quantitation (2 ng/ml); NC, not calculable due to low concentrations; NA, not applicable.
| Study type | Dose (mg/kg/day) | Pregnant females | Maternal findings | Developmental findings |
|------------|-----------------|-----------------|-------------------|-----------------------|
|            | Amodiaquine | Artesunate |                  |                       |
| **DRF1**   | 3              | 1              | 10                | Decreased body weight gain during gestation |
|            | 10             | 4              | 10                | Decreased food consumption and body weight gain during gestation |
|            | 20             | 8              | 8                 | -                     |
|            | 30             | 12             | 8                 | 4 of 8 females had total litter loss prior to first observation on PPD 1 |
| **DRF2**   | 4              | -              | 10                | Decreased body weight gain during the first 3 days of dosing |
|            | 12             | -              | 9                 | Decreased food consumption and body weight gain during gestation |
|            | 36             | -              | 9                 | Decreased food consumption and body weight gain during gestation and decreased body weight gain during 1st week of lactation |
|            | -              | 1.6            | 10                | -                     |
|            | -              | 4.8            | 10                | -                     |
|            | -              | 14.4           | 10                | Decreased body weight gain late in gestation likely related to death of conceptuses |
| **PPN**    | 3              | 1              | 27                | mNOEL dNOEL           |
|            | 10             | 4              | 25                | 19% decrease in body weight gain GD 6 to 9 and 3% decrease GD 6 to 20 |
|            | 20             | 8              | 23                | 51% decrease in body weight gain GD 6 to 9 and 17% decrease GD 6 to 20; decreased food consumption during gestation and 1st 2 weeks postpartum |
|            | 20             | -              | 27                | 45% decrease in body weight gain GD 6 to 9 and 12% decrease GD 6 to 20; decreased food consumption during gestation and 1st 2 weeks postpartum |
|            | -              | 8              | 27                | 74% decrease in body weight gain GD 12 to 20 likely largely related to death of conceptuses |
| **GD**, gestational day; **DRF**, dose-range-finding study; **PPN**, pre- and postnatal development study; **PPD**, postpartum day; **dNOEL**, developmental no effect level; **mNOEL**, maternal no effect level. |
mg/kg/day artesunate alone or combinations of amodiaquine + artesunate (3 + 1.2, 10 + 4, and 22.5 + 9 mg/kg/day, respectively; Table 6).

The group treated with 22.5 mg/kg/day amodiaquine alone had 28 to 43% decreases in mean food consumption during the dosing period and a 68 g loss in mean body weight from GD 6 to 9 compared with a 30 g mean gain in the control. There were no developmental effects in this group. Thus, the dNOEL for amodiaquine administered alone was 22.5 mg/kg/day (HED = 7 mg/kg/day).

In the group treated with 9 mg/kg/day artesunate alone, 11 of 20 females aborted between GD 20 and 25. Among the pregnant females that survived to cesarean section on GD 29, there was a marked effect on embryo survival apparent as a mean postimplantation loss of 57% compared with 6% in the control group. Three fetuses from two of the seven litters evaluated for visceral alterations had treatment-related cardiovascular malformations. There was also an 8% decrease in mean fetal weight. Mean body weight remained the same between GD 15 and 19 compared with a 63 g gain in the control group during this period which was at least partially attributable to the death of conceptuses.

The group receiving both of these treatments (22.5 mg/kg/day amodiaquine plus 9 mg/kg/day artesunate) had less of an effect on embryo survival compared with the 9 mg/kg/day artesunate alone group with only three aborted litters and a mean postimplantation loss among those mothers surviving to cesarean section of 37%. There were also maternal effects in this group consisting of a 72 g loss in mean body weight from GD 6 to 9 compared with a 30 g mean gain in the control. There were no maternal or developmental effects in the other groups receiving both amodiaquine and artesunate (3 + 1.2 mg/kg/day and 10 + 4 mg/kg/day, respectively).

In the rabbit EFD study, plasma concentrations of amodiaquine, desethylamodiaquine (desethylAQ), artesunate, and DHA were measured on GD 6 and 18. Toxicokinetic data are shown in Table 7.

### THERAPEUTIC DOSING REGIMEN

Amodiaquine is commonly administered as part of a combination product with artesunate (Coarsucam) in a ratio of 1 part artesunate to 2.7 parts amodiaquine. The recommended therapeutic dosing regimen is 4 mg/kg/day artesunate and 10 (7.5 to 15) mg/kg/day amodiaquine once daily for 3 days (WHO, 2015a).

### CLINICAL PHARMACOKINETICS

Pharmacokinetic data following the administration of 10 mg/kg/day amodiaquine once daily for 3 days to pregnant women in the second and third trimester are shown in Table 9 (Rijken et al., 2011). The active metabolite, desethylamodiaquine, has a much longer half-life (10 days) than amodiaquine (12.4 hr). AUC0-24h values were calculated following the third (last) dose (Table 9) and were likely the highest values for any 24-hr period.

### EXPERIENCE IN PREGNANT WOMEN IN THE FIRST TRIMESTER

Among 11 women with a gestational age of ≤20 weeks treated with amodiaquine for malaria in the first trimester, all delivered full term babies of normal birth weight and no congenital anomalies (Mosha et al., 2014).

### ASSESSMENT

There is only limited published experience concerning the safety of amodiaquine in the first trimester. In rats and rabbits, amodiaquine did not cause embryo death or malformations. Decreased fetal weight and delayed ossification were noted in rats but only at a dose (30 mg/kg/day) that caused maternal toxicity so there was no observation of selective developmental effects. The HED ratios in the rat and rabbit EFD studies were 0.2 and 0.7, respectively, and 0.05 in the rat PPN study. The HED ratio at 30 mg/kg/day in the rat EFD study, which caused only minor embryonal effects, was 0.5.

An important question for a study in which a combination of compounds is administered is whether the compounds interact synergistically to produce embryotoxicity that is worse than that with either agent alone. This was not seen. In fact, the opposite effect was observed in both...
species with the coadministration of 30 mg/kg/day amodiaquine seeming to provide some protection against the embryotoxicity induced by 12 mg/kg/day artesunate in the rat EFD study, 20 mg/kg/day amodiaquine seeming to provide some protection against the embryotoxicity of 8 mg/kg/day artesunate in the rat PPN study, and 22.5 mg/kg/day amodiaquine seeming to provide some protection against the embryotoxicity of 9 mg/kg/day artesunate in the rabbit EFD study.

The values for Cmax and AUC0-24h for amodiaquine and desethylamodiaquine at the therapeutic dose in humans and at the dNOELs in rats and rabbits are shown in Tables 9 and 10, respectively. The Cmax and AUC ratios were less than 1 for both rat studies (EFD and PPN), for both GDs when plasma concentrations were measured (GD 6 and 17) and for both analytes (amodiaquine and desethylamodiaquine). There were only minor effects at 30 mg/kg/day in the rat EFD study and the Cmax and AUC ratios for amodiaquine at that dose were 0.6 and 0.3, respectively, based on GD 6 measurements and 1.8 and 3.0, respectively, based on GD 17 measurements. The Cmax and AUC ratios for desethylamodiaquine at that dose were both 0.6 based on GD 6 measurements and 0.7 and 0.9, respectively, based on GD 18 measurements.

To calculate a single AUC ratio based on the combined exposure to both amodiaquine and desethylamodiaquine, the assumption was made that any embryotoxic activity was related to the antimalarial activity of the compound. The in vitro antimalarial activity of amodiaquine and desethylamodiaquine varied among four strains of *P. falciparum* but amodiaquine was consistently more potent (Childs et al., 1989; Mariga et al., 2005). For the purpose of calculating an AUC0-24h for antimalarial activity, it was assumed that amodiaquine was approximately 2.5-fold more potent than desethylamodiaquine and the AUC was expressed as “amodiaquine equivalents” for both the dNOELs in animals and for exposure at the therapeutic dose in pregnant women (Tables 10 and 9, respectively). The AUC ratios based on these estimates were 0.02 and 0.2 for the rat on GD 6 and 17, respectively, and, for the rabbit, 0.11 on both GD 6 and 18. At 30 mg/kg/day in the rat (a minor effect level), the AUC ratios based on antimalarial activity were 0.1 and 1 on GD 6 and 17, respectively. Regardless of how they are calculated, several of the exposure ratios for amodiaquine in rats and rabbits are relatively low.

Overall, there were no unusual findings related to the administration of amodiaquine during organogenesis in rats and rabbits. The induction of embryo death and malformations seen with the combination of amodiaquine and artesunate was attributable to artesunate. There was no evidence of a synergistic interaction between amodiaquine

### Table 10. Amodiaquine: Exposure at dNOEL in Animal Studies with Treatment during Organogenesis

| Species | Study | Dose (mg/kg/day) | HED (mg/kg/day) | Developmental effects | Gestational day | Cmax (ng/ml) | AUC0-24h (ng.h/ml) | Cmax (ng/ml) | AUC0-24h (ng.h/ml) | Cmax (ng/ml) | AUC0-24h (ng.h/ml) |
|---------|-------|-----------------|-----------------|----------------------|----------------|-------------|------------------|-------------|------------------|-------------|------------------|
| Rat     | EFD   | 10            | 1.6             | None                 | 6              | BLQ        | NC           | 15.2        | 130              | 6.1         | 52               |
|         |       | 17            | 7.7             | NC                   | 15.2           | 130         | 6.1         | 52          |                  |             |                  |
|         |       | 30            | 16.8            | 64.5                 | 36.5           | 467         | 31           | 251         |                  |             |                  |
| PPN*    | 3     | 0.5           | None            | 138                   | 1187           | 222         | 1187         | 222         | 1187            | 1187        | 1187            |
|         | 17    | 54.4          | 609             | 235                   | 5116           | 148         | 2655         | 148         | 2655            |             |                  |
| Rabbit  | EFD   | 22.5          | 7               | None                 | 138           | 1187        | 222         | 1187        | 222             | 1415        |
|         | 18    | 82.2          | 745             | 258                   | 4915           | 185         | 2711         | 185         | 2711            |             |                  |

*a*dNOEL from PPN study; toxicokinetic data from the rat EFD study.

*b*Coadministered with 4 mg/kg/day artesunate.

*c*Coadministered with 1 mg/kg/day artesunate.

*d*Calculated by dividing desethylAQ value by 2.5 and adding the dividend to the AQ value.

EFD, embryo-fetal development study; PPN, pre- and postnatal development study; dNOEL, developmental no effect level; HED, human equivalent dose; AQ, amodiaquine; EQ, equivalents; BLQ, below the lower limit of quantitation (10 ng/ml); NC, not calculable due to low concentrations.
and artesunate for the production of these effects. To the contrary, incidences of embryo death were lower in the amodiaquine + artesunate combination groups. Most of the estimated exposure ratios for rats and rabbits following treatment throughout organogenesis were \( \leq 1.1 \).

## Lumefantrine

### STUDIES IN PREGNANT ANIMALS

ICH- and GLP-compliant EFD studies of lumefantrine, artemether, and 1:6 combinations of artemether and lumefantrine were conducted in rats and rabbits with treatment throughout organogenesis (Clark et al., 2016). Study details for the studies involving lumefantrine are provided in Table 11. Both EFD studies of lumefantrine had a high dose of 1000 mg/kg/day which is generally considered to be a limit dose. In the rat, there was a decrease in litter size at 1000 mg/kg/day but no developmental effects at 300 mg/kg/day (dNOEL; HED = 48 mg/kg/day). In the rabbit, there were no developmental effects even at the limit dose of 1000 mg/kg/day (dNOEL; HED = 323 mg/kg/day). At that dose, the \( C_{\text{max}} \) and AUC\(_{0-24h} \) values following the last dose on GD 19 were 38 \( \mu \text{g/ml} \) and 592 \( \mu \text{g.h/ml} \), respectively.

When artemether and lumefantrine were coadministered, the developmental effects observed were largely as would have been expected with the administration of artemether alone. Also, the dNOELs for artemether when administered as part of the combination (4.3 and 15 mg/kg/day for rats and rabbits, respectively; Table 11) were similar to those when artemether was administered alone (3 and 25 mg/kg/day, for rats and rabbits, respectively).

### THERAPEUTIC DOSING REGIMEN

Lumefantrine is used in combination with artemether. For a 50-kg human, the dosing regimen is 480 mg lumefantrine administered twice daily (with 80 mg artemether) for 3 days corresponding to daily doses for a 50-kg person of 19.2 mg/kg/day lumefantrine and 3.2 mg/kg/day artemether (WHO, 2015a).

### CLINICAL PHARMACOKINETICS

In two pharmacokinetic studies, pregnant women were administered the standard therapeutic dose of artemether+lumefantrine at 0, 8, 24, 36, 48, and 60 hr. In the first study (McGready et al., 2006), blood sampling began at 60 hr and the \( C_{\text{max}} \) and AUC\(_{0\rightarrow\infty} \) values for lumefantrine were determined to be 7.3 \( \mu \text{g/ml} \) and 252 \( \mu \text{g.h/ml} \), respectively. In the second pharmacokinetic study (Tarning et al., 2013), blood sampling started at the first dose and extended to 168 hr after the last dose. The \( C_{\text{max}} \) and AUC\(_{48\rightarrow72h} \) values for lumefantrine were estimated to be 9.2 \( \mu \text{g/ml} \) and 170 \( \mu \text{g.h/ml} \), respectively (Clark et al., 2016).

## EXPERIENCE IN PREGNANT WOMEN IN THE FIRST TRIMESTER

Studies in which the safety of lumefantrine in the first trimester was assessed have involved the administration of the combination of lumefantrine with artemether. The safety of the artemether+lumefantrine combination in pregnancy has been reviewed (Manyando et al., 2012; Mutabingwa and Adam, 2013; Visser et al., 2014). There was a total of 456 first trimester exposures to just artemether+lumefantrine reported in five articles (Adam et al., 2009, \( N = 3 \); Manyando et al., 2010, 2015, \( N = 150 \); Rulisa et al., 2012, \( N = 139 \); Mosha et al., 2014, \( N = 164 \)). All women were infected with malaria except for 43 women in Rulisa et al., 2012. In Manyando et al., 2010, 4 of 150 malaria patients receiving only artemether+lumefantrine in the first trimester aborted compared with 0 of 129 patients receiving sulfadoxine+pyrimethamine and/or quinine. However, the background rate was reported to be 12 to 16%.

It was suggested that the lower rate of abortion observed in this study was attributable to the enrollment of women primarily after the first trimester so early abortions would not necessarily have been included. In Rulisa et al. (2012), more obstetric complications were observed in the group with malaria and treated with artemether+lumefantrine compared with the group of pregnant women without malaria and not treated with artemether+lumefantrine. It was not clear whether the increased incidence of obstetric complications was related to malaria or the treatment with artemether+lumefantrine. These studies did not reveal an effect of artemether+lumefantrine on the miscarriage rate or incidence of malformations.

A meta-analysis of data from six articles was conducted recently to evaluate the effects of artesunate or ACTs administered during the first trimester (Dellicour et al., 2017). There were no detectable differences between the group treated with artemisinin or ACT (\( n = 717 \) for evaluation of the miscarriage rate and \( n = 551 \) for major congenital anomalies) compared with the group treated with quinine (\( n = 947 \) and 741, respectively). The studies evaluated included Manyando et al., 2010, Rulisa et al., 2012, and Mosha et al., 2014, and 511 of the 717 women in the artemisinin plus ACT group evaluated for the miscarriage rate and at least 501 of the 551 evaluated for major congenital anomalies had been treated with artemether+lumefantrine.

### ASSESSMENT

For lumefantrine administered alone, the HED ratios were 2.5 for rat and 17 for rabbits and the \( C_{\text{max}} \) and AUC ratios for rabbit were 4.6 and 3.5. These are much more favorable exposure ratios than those for all of the other antimalarials discussed in this article. Also, there are far more clinical data in the first trimester for artemether+lumefantrine (\( N > 456 \)) than for any other ACT and no effects of...
treatment on the rates of miscarriage or congenital malformations have been observed.

**Mefloquine**

**HALF-LIFE OF ELIMINATION FOR MELOQUINE**

Mefloquine has a long half-life for elimination in humans (9 to 21 days; Desjardins et al., 1979; Na-Bangchang et al., 1999; Simpson et al., 1999) as well as in rats (69 hr; A. Kistler, unpublished data), rabbits (69 and 83 hr in two independent determinations; A. Kistler, unpublished data), and mice (35 hr; A. Kistler, unpublished data). The therapeutic dosing regimen for mefloquine is up to 25 mg/kg administered within a period of up to approximately 48 hr (see section below on Therapeutic Dosing Regimen) which is a relatively short period compared with the dosing period in standard EFD studies. Due to the long half-life,

| TABLE 11. Lumefantrine: Summary of Results of Definitive EFD Studies in Rats and Rabbits |
|-----------------------------------------------|-----------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Species | Study designation | Treatment period | Group size* | GD of C-section | Dose (mg/kg/day) | Maternal findings | Developmental findings |
|---------|--------------------|------------------|-------------|-----------------|-----------------|-----------------|-----------------|
| Rat 1   | GD 6-17            | 24               | 20          | Lum Am Am + Lum | 100             | None            | None            |
|         |                    |                  |             |                 | 300             | None            | None (dNOEL)    |
|         |                    |                  |             |                 | 1000            | 24% decrease in body weight gain from GD 13-18 due to 4 small litters | 25% decrease in litter size |
|         |                    |                  |             |                 |                 | None            | None            |
|         | 3                  | GD 6-15          | 26          | 20              | 25.7            | None            | None (dNOEL)    |
|         |                    |                  |             |                 | 85.7            | Decreased body weight gain due to total litter loss | Total litter loss except for one litter with 2 live fetuses |
|         |                    |                  |             |                 | 257.1           | Decreased body weight gain due to total litter loss | Total litter loss in all dams |
| Rabbit 4| GD 7-19            | 20               | 29          | 100             | 500             | None            | None            |
|         |                    |                  |             |                 | 1000            | 7 to 15% decreases in food consumption from GD 7-16 | None (dNOEL) |
|         | 6                  | GD 7-19          | 20          | 29              | 30              | None            | None            |
|         |                    |                  |             |                 | 90              | None            | None            |
|         |                    |                  |             |                 | 150             | 2 does had blood in pan from GD 18 to 20 or 23 | 5 does with low implant count including 2 does with total litter loss (with blood in pan) and 2 abortions on GD 22 |

From Clark et al., 2016.

*Number assigned to group.

EFD, embryo-fetal development study; GD, gestational day; AM, artemether; Lum, lumefantrine; dNOEL, highest no developmental effect level.
| Study No. | Species/strain | Treatment period/C-section | Dose | Litters evaluated | Maternal findings | Developmental findings |
|----------|----------------|---------------------------|------|-------------------|-------------------|-----------------------|
| M1       | Rat, Sprague-Dawley | GD 6-15/ GD 20 | 10 mg/kg/day | 17 | - | - |
|          |                 |                           |      |                   | 6 deaths among pregnant females; decreased food consumption and body weight gain after beginning of treatment | |
|          |                 |                           |      |                   | 32% decrease in fetal weight; increased incidences of anomalies including 50 fetuses with domed skull and 13 fetuses with hydrocephalus compared to none in control | |
| M2       | Rat, Füllinsdorf albino | GD 6, 9, 12/ GD 20 | 100/50 mg/kg/day | 18/7 | 11/12 | mNOEL | dNOEL |
|          |                 |                           |      |                   | 19% decrease in body weight gain during the treatment period | |
|          |                 |                           |      |                   | 23% decrease in litter size due to decreased implants (6.3 implants/litter = 56% of 11.3 corpora lutea (CLs)/litter) compared to control (7.8 implants/litter = 66% of 11.9 CLs/litter) | |
| M3       | Rabbit, Swiss hare | GD 6, 9, 12, 15/ GD 29 | 30/15 mg/kg/day | 19 | - | - |
|          |                 |                           |      |                   | 37% decrease in body weight gain during the treatment period | |
|          |                 |                           |      |                   | Statistically significant (p < 0.05) increased resorption rate (33.8%) compared to control (22.4%) | |

**TABLE 12.** Mefloquine: Oral EFD Studies in Pregnant Rats and Rabbits
daily dosing in laboratory animals results in accumulation and steady state (as estimated by five half-lives) is reached after approximately 14, 16, and 7 days for rats, rabbits, and mice, respectively.

Some of the animal studies described below used dosing regimens other than daily dosing at constant dose. These other regimens were designed to achieve less day-to-day variation in exposure. Each of these regimens involved the administration of a loading dose on the first day of dosing followed subsequently by the administration of multiple doses equal to one half the initial dose with intervals of 3 days (rats and rabbits) or 2 days (mice). Based on the indicated half-lives, it is estimated that the exposure during each 24-hr period of the intended treatment period was always more than half of that following the first dose.

**STUDIES IN PREGNANT ANIMALS**

Regulatory-compliant, GLP EFD studies. A series of developmental toxicity studies with varying periods of treatment with mefloquine, including throughout organogenesis, were conducted in rats, rabbits, and mice under the sponsorship of Roche. Also, a study was conducted in pregnant cynomolgus monkeys with single doses administered on 2 days of gestation, GD 30 and 117. Mefloquine (molecular weight 378.3) was administered in these animal studies as the hydrochloride salt (molecular weight 414.8; factor 1.10). Doses are expressed as mg of the salt per kilogram body weight per day.

**Rat studies.** Two developmental toxicity studies were conducted in rats (Studies M1 and M2). Study design details and a summary of results are shown in Table 12. In Study M1 (T. Short, unpublished data; Minor et al., 1976), mefloquine HCl was administered daily during organogenesis (GD 6 through 15). It is estimated, based on a half-life of elimination of approximately 69 hr in rats (A. Kistler, unpublished data), that the exposure gradually increased following each dose and the exposure during the 24 hr following the last dose was approximately 4.2-fold greater than that following the first dose.

In the second study (Study M2), doses were administered on GD 6, 9, and 12 to reduce accumulation of drug that would occur with daily administration. At each dose level, the dose on GD 6 was twice that on GD 9 and 12. Based on the indicated half-life, it is estimated that the exposure during each 24-hr period of the intended treatment period was always more than half of that following the first dose.

At the maternally lethal dose of 100 mg/kg/day in Study M1, there were increased incidences of fetal anomalies including domed skull and hydrocephalus. The middle dose group in that study (20 mg/kg/day) was both the mNOEL and the dNOEL (HED < 3.2 mg/kg/day).

In Study M2, the highest dose level (dosing at 500 mg/kg followed by 250 mg/kg) caused maternal deaths and

| Study No. | Species/strain | Dose | Treatment period/ C-section | Maternal findings | Developmental findings |
|-----------|----------------|------|-----------------------------|-------------------|------------------------|
| M1        | Rabbit         | 160/80 | GD 6, 9, 12, 15/GD 29 | 78% decrease in body weight gain | Increased resorption rate (41.1%) compared to control (22.4%); Low incidences of malformations including 2 fetuses with gastroschisis |
| M2        | Swiss hare     | 80/40 | GD 6, 9, 12, 15/GD 29 | 50/25/12 mNOEL | Increase in resorption rate (14.0%) compared to control (10.0%) |

| Table 12. Continued |
|---------------------|
| Species/strain      | Treatment period/C-section |
|---------------------|-----------------------------|
| Rat                 | GD 6, 9, 12, 15/GD 29       |
| Rabbit              | GD 6, 9, 12, 15/GD 29       |

aThe day evidence of mating was found (rat) or the day of mating (rabbit) = Day 0 of gestation.

bNumber of litters examined externally (E), viscerally (V), skeletally (S), or allowed to deliver (DEL).
the middle dose level (dosing at 250 mg/kg followed by 125 mg/kg) was associated with a 19% decrease in maternal body weight gain and a 23% decrease in mean litter size related to a decrease in mean implant count. Low implant counts such as these are considered to result from embryonic deaths so early in gestation that no implantation site is visible at cesarean-section unless the uterus has been stained with ammonium sulfide (see Salewski, 1964; Schumacher et al., 1968; Staples, 1971). The low dose group in that study (dosing at 100 mg/kg followed by 50 mg/kg) was both the mNOEL and the dNOEL. Taking into account the intermittent dosing, the HED at the dNOEL is considered to be ~4 mg/kg/day.

**Rabbit studies.** Two EFD studies were conducted in rabbits (Studies M3 and M4). Study design details and a summary of results are shown in Table 12. In both studies, doses were administered on GD 6, 9, 12, and 15 to reduce accumulation of drug that would occur with daily administration. At each dose level, the dose on GD 6 was twice that on subsequent days. In Study M4, a single dose level was tested (80 mg/kg on GD 6/40 mg/kg on subsequent days) to further evaluate findings of questionable relationship to treatment at the same dose level in Study M3. Based on an estimated half-life of elimination in rabbits of 76 hr (mean of two independent determinations of 69 and 83 hr reported in A. Kistler, unpublished data), it is estimated that the exposure during each 24-hr period between GD 6 and 18 was between 65% and 103% of that following the first dose.

There was marked maternal toxicity including possibly treatment-related deaths at the high dose in Study M3. In both Studies M3 and M4, there were increased resorption rates seen in the group treated with 80 mg/kg on the first day of dosing and 40 mg/kg on subsequent days. Decreased maternal body weight gain was seen at that dose in Study M3 but not in Study M4. The dNOEL was the low dose in Study M3 (dosing at 30 mg/kg followed by 15 mg/kg) and the HED at the dNOEL (taking into account the intermittent dosing) is considered to be ~2 mg/kg/day.

**Mouse studies.** Two EFD studies were conducted in mice (Studies M5 and M6). Study design details and a summary of results are shown in Table 13.

In Study M5 (T. Short unpublished data; Minor et al., 1976), mefloquine HCl was administered at doses of 10, 20, 100, and 200 mg/kg/day daily during organogenesis (GD 6 through 15) and at 150 mg/kg/day for 3-day periods (GD 9 to 11 and GD 12 to 14). It is estimated, based on a half-life of elimination of approximately 35 hr in mice (A. Kistler, unpublished data), that the exposure gradually increased following each dose and that, for the groups treated on GD 6 to 15, the exposure during the 24 hr following the last dose was approximately 2.6-fold greater than that following the first dose. No maternal effects were observed in the groups treated at ≤100 mg/kg/day on GD 6 to 15 and in the two groups treated with 150 mg/kg/day for 3-day periods (GD 9 to 11 and GD 12 to 14).

Maternal deaths, decreased maternal body weight gain, and increased embryonic resorptions were observed only in the group treated on GD 6–15 at 200 mg/kg/day. Treatment-related incidences of cleft palate were observed at 200 mg/kg/day as well as in the absence of maternal toxicity in the two groups treated with 150 mg/kg/day for 3-day periods (GD 9 to 11 and GD 12 to 14). Decreases in fetal weight occurred in the groups receiving 100 and 200 mg/kg/day throughout organogenesis and the group treated with 150 mg/kg/day during GD 9 to 11. The dNOEL was 20 mg/kg/day (HED = 1.6 mg/kg/day). Thus, mefloquine was selectively developmentally toxic in mice because it caused developmental effects in the absence of maternal toxicity: cleft palate when administered at 150 mg/kg/day from GD 9 to 11 or GD 11 to 14 and decreases in fetal weight when administered at 100 mg/kg/day from GD 6 to 15 and at 150 mg/kg/day from GD 9 to 11. The induction of cleft palate by mefloquine was not observed in rats and rabbits. Mice are particularly sensitive to the induction of cleft palate.

In the second mouse study (Study M6), doses were administered on GD 6, 8, 10, 12, and 14 to reduce the accumulation of drug that would occur with daily administration. At each dose level, the dose on GD 6 was twice that on subsequent days. Based on the indicated half-life, it is estimated that the exposure during each 24-hr period between GD 6 and 16 was between 51% and 100% of that following the first dose. Decreases in maternal body weight gain were observed at all doses. In the 160/80 and 400/200 mg/kg/day dose groups, there were maternal deaths, increased incidences of embryonic resorptions and cleft palate, and decreases in fetal weight. The dNOEL was the dose level which received 80 mg/kg on the first day of dosing and 40 mg/kg on subsequent days. Taking into account the intermittent dosing, the HED is considered to be ~2 mg/kg/day.

**MONKEY STUDY WITH DOSING ON GD 30 AND 117**

A study was conducted in which one group of 11 pregnant cynomolgus monkeys was treated orally on GD 30 and 117 with 20 mg/kg mefloquine HCl (Study M7; I. Osterburg, unpublished data; see Table 13). There was no concurrent control group and the results were compared with historical control data in the laboratory. The uterine contents were removed by cesarean section on GD 150. In two of the mothers, the conceptus had resorbed starting between approximately GD 30 and 45 but these resorptions were not considered treatment-related. Nine live fetuses were weighed, examined externally, and necropsied, including the weighing of selected organs. The carcasses were cleared and the skeletons were stained and...
| Study no. | Species/strain | Treatment period<sup>1<sub>¥</sub></sup> | Dose | Litters evaluated | Maternal findings | Developmental findings |
|-----------|----------------|----------------------------------|------|-------------------|-------------------|------------------------|
| M5        | Mouse, CD-1    | GD 6-15/ GD 18                   | 10   | 18                | -                 | -                      |
|           |                |                                  | 20   | 17                | mNOEL             | dNOEL                 |
|           |                |                                  | 100  | 17                | Decreased fetal weight | 35% resorption rate compared to 11% in controls; 23% decrease in fetal weight; 8 fetuses with cleft palate and 4 fetuses with hydrocephaly from a total of 3 litters compared to none in concurrent control |
|           |                |                                  | 200  | 9                 | 160/80            | 10 deaths; 24% decrease in body weight gain between GD 6 and 16. At c-section, there was a 23.8% resorption rate compared to 9.5% in control; 20% decrease in mean live fetal weight; 5 fetuses from 3 litters with cleft palate and 1 fetus with exencephaly compared to none in control. |
| M6        | Mouse, Füllinsdorf albino | GD 6, 8, 10, 12, 14/ GD 18 | 80/40 | 15/10/20 | 17% decrease in body weight gain between GD 6 and 16. | dNOEL |
|           |                |                                  | 160/80 | 18/11/14 | 3 deaths; 24% decrease in body weight gain between GD 6 and 16. At c-section, there was a 16.3% resorption rate compared to 9.5% in control; 8.7% decrease in mean live fetal weight; 1 fetus with cleft palate compared to none in the control. |
|           |                |                                  | 400/200 | 18/11/2  | 10 deaths; 29% decrease in body weight gain between GD 6 and 16. At c-section, there was a 23.8% resorption rate compared to 9.5% in control; 20% decrease in mean live fetal weight; 5 fetuses from 3 litters with cleft palate and 1 fetus with exencephaly compared to none in control. In the natural delivery subgroup, there was a 46% decrease in live litter size on Day 1 postpartum. |
examined. There were no treatment-related abortions or fetal findings. The HED at the dNOEL was 6 mg/kg.

PREGNANT RAT STUDIES WITH DOSING ON GD 9 TO 11 AND COADMINISTRATION WITH ARTESUNATE

Two studies in Wistar rats (M8 and M9) were conducted with the same treatment regimens on GD 9 to 11 and treatment groups receiving oral doses of three mefloquine hydrochloride alone, artesunate alone, and combinations of mefloquine hydrochloride and artesunate (Boareto et al., 2012, 2013). Study design details and a summary of results are shown in Table 14. It is estimated, based on a half-life of elimination for mefloquine in rats of approximately 69 hr (A. Kistler, unpublished data), that the exposure gradually increased following each dose of mefloquine alone and the exposure during the 24 hr following the last dose was approximately 2.4-fold greater than that following the first dose.

In Study M8, all fetuses were removed on GD 20, weighed and examined externally, one-third of the fetuses were examined viscerally and two-thirds skeletally. When mefloquine was administered alone, there were treatment-related resorptions at 80 mg/kg/day. The mNOEL and dNOEL were both 30 mg/kg/day (HED 5 5 mg/kg/day). When administered alone, artesunate caused the expected effects including bent scapula and forelimb bones (e.g., 29.8% of fetuses with bent humerus) at 15 mg/kg/day and 100% embryo death at 40 mg/kg/day. The coadministration of mefloquine had an ameliorative effect because there were no fetuses with bent scapula or forelimb bones in the 30/15 mg/kg/day mefloquine/artesunate group and four litters survived following treatment with 80/40 mg/kg/day mefloquine/artesunate.

In Study M9, all embryos were removed on GD 12 and examined externally and five or six per group were examined histologically. No effects were observed in either group treated with mefloquine alone (30 and 80 mg/kg/day). The expected effects (dead implants and anemic yolk sacs) were observed in the groups treated with 15 or 40 mg/kg/day artesunate alone. The coadministration of mefloquine may have had an ameliorative effect because there were no dead implants and anemic yolk sacs in the 30/15 and 80/40 mg/kg/day mefloquine/artesunate group and four litters survived following treatment with 80/40 mg/kg/day mefloquine/artesunate.

In Study M10, an embryofetal study was conducted in Wistar rats (El-Dakdoky, 2015). Groups of 9 or 10 pregnant females were treated on GD 1, 6, or 13 with single oral doses of 45 or 187 mg/kg mefloquine hydrochloride. Study design details and a summary of results are shown in Table 14. The doses were selected based on kinetic studies in 7-week-old nonpregnant female Sprague-Dawley rats (Dow et al., 2006). Single oral doses of 45 and 187 mg/kg mefloquine had been found to

| Study no. | Species/strain   | Treatment period/ C-section | Dose | Maternal findings | Developmental findings |
|-----------|------------------|------------------------------|------|-------------------|-----------------------|
| M7        | Cynomolgus monkey | GD 30 and 117/GD 150 ± 1   | 6    | Group compared to historical controls; no effects noted |

| Study no. | Species/strain   | Treatment period/ C-section | Dose | Maternal findings | Developmental findings |
|-----------|------------------|------------------------------|------|-------------------|-----------------------|
| M8        | Wistar rat       | GD 9 and 11                  | 20   | Pregnant females  | dNOEL                 |

| Study no. | Species/strain   | Treatment period/ C-section | Dose | Maternal findings | Developmental findings |
|-----------|------------------|------------------------------|------|-------------------|-----------------------|
| M9        | Wistar rat       | GD 9 and 11                  | 20   | Pregnant females  | dNOEL                 |

| Study no. | Species/strain   | Treatment period/ C-section | Dose | Maternal findings | Developmental findings |
|-----------|------------------|------------------------------|------|-------------------|-----------------------|
| M10       | Wistar rat       | GD 1, 6, or 13               | 45/187 | Single oral doses of 45 or 187 mg/kg mefloquine hydrochloride. Study design details and a summary of results are shown in Table 14. The doses were selected based on kinetic studies in 7-week-old nonpregnant female Sprague-Dawley rats (Dow et al., 2006). Single oral doses of 45 and 187 mg/kg mefloquine had been found to

| Study no. | Species/strain   | Treatment period/ C-section | Dose | Maternal findings | Developmental findings |
|-----------|------------------|------------------------------|------|-------------------|-----------------------|
| M10       | Wistar rat       | GD 1, 6, or 13               | 45/187 | Single oral doses of 45 or 187 mg/kg mefloquine hydrochloride. Study design details and a summary of results are shown in Table 14. The doses were selected based on kinetic studies in 7-week-old nonpregnant female Sprague-Dawley rats (Dow et al., 2006). Single oral doses of 45 and 187 mg/kg mefloquine had been found to
| Study no. | Species/strain | Treatment period/ C-section | Mq (mg/kg) | As (mg/kg) | Litters evaluated | Maternal findings | Developmental findings | Reference |
|-----------|----------------|-----------------------------|------------|------------|------------------|------------------|----------------------|-----------|
| M8        | Rat, Wistar    | GD 9 to 11/ GD 20<sup>a,c</sup> | 30         | -          | 19               | mNOEL            | cNOEL                | Boareto et al., 2012 |
|           |                |                             | 80         | -          | 19               | 50% decrease in maternal body weight gain from GD 9 to 12 | 1 totally resorbed litter compared to none in control; 23% decrease in litter size due largely to 22% decrease in implant count |
|           |                |                             | -          | 15         | 20               | mNOEL            | 5 totally resorbed litters; no effect on implant count or litter size in litters with live fetuses; treatment-related skeletal malformations - % of fetuses (compared to none in control): 15.4% bent scapula, 29.8% bent humerus, 6.4% bent radius, 19.2% bent ulna; also 55.6% wavy ribs compared to 12.3% in control |
|           |                |                             | -          | 40         | 19               | Decreased body weight gain due to whole litter resorptions | 19 (all) totally resorbed litters |
|           |                |                             | 30         | 15         | 19               | mNOEL            | 5 totally resorbed litters; no effect on implant count or litter size in litters with live fetuses; 57% fetuses with wavy rib compared to 12.3% in control; no fetuses with bent scapulas or forelimb bones |
|           |                |                             | 80         | 40         | 18               | 93% decrease in maternal body weight gain from GD 9 to 12 | 14 totally resorbed litters; no effect on implant count or litter size in litters with live fetuses; no fetuses with bent scapulas or forelimb bones |
| Study no. | Species/strain | Treatment period/ C-section* | Dose (mg/kg) | Litters evaluated | Maternal findings | Developmental findings | Reference |
|-----------|----------------|-----------------------------|--------------|-------------------|------------------|-----------------------|-----------|
| M9        | Rat, Wistar    | GD 9 to 11/ GD 12<sup>a,b</sup> | 0            | 0                 | 12               | -                     | Boareto et al., 2013 |
|           |                |                             | 30           | -                 | 13               | -                     |           |
|           |                |                             | 80           | -                 | 12 mNOEL         | dNOEL                 |           |
|           |                |                             | -            | 15                | 14               | -                     |           |
|           |                |                             | -            | 40                | 13 mNOEL         | 40% dead implants, 77% anemic yolk sacs |           |
|           |                |                             | 30           | 15                | 12               | -                     |           |
|           |                |                             | 80           | 40                | 13 mNOEL         | 33% dead implants, 46% anemic yolk sacs |           |
| M10       | Rat, Wistar    | Single days of dosing on GD 1, 6 or 13/ GD 21<sup>a,d</sup> | 45           | 10                | Signs of illness (?) | Possible increased incidences of fetuses with dilated cerebral ventricle, all treatment days | El-Dakdoky, 2015 |
|           |                |                             | 187          | 9 or 10           | Decreased maternal body weight gain | 11 to 21% decreases in fetal weight with all treatment days; possibly increased incidences of fetuses with dilated cerebral ventricle with all treatment days |         |

*The day evidence of mating was found = Day 0 of gestation.
<sup>a</sup>The last dose exposure multiple for 3 days of dosing in rats is 2.4.
<sup>b</sup>One third of fetuses examined viscerally and two-thirds skeletally.
<sup>c</sup>Half of fetuses examined viscerally and half skeletally.
GD, gestational day; Mq, mefloquine; As, artesunate; mNOEL, maternal no effect level; dNOEL, developmental no effect level.
generate plasma concentrations at 72 hr postdose (the only time point) that were comparable to those in humans following prophylactic dosing (~800 ng/ml) and malarial treatment (~2100 ng/ml; Simpson et al., 1999), respectively.

In Study M10, all fetuses were weighed and examined externally and half of the fetuses were examined viscerally and half skeletally. Increased resorptions were not observed in any treatment group. Decreased fetal weight was observed in all groups treated with 187 mg/kg mefloquine. There were possibly increased incidences of fetuses with dilated cerebral ventricles following treatment at both 45 and 187 mg/kg/day and on all treatment days. Thus, there was no clear dNOEL.

THERAPEUTIC DOSING REGIMEN

The adult dose recommended by the Centers for Disease Control and Prevention (CDC) for uncomplicated malaria is 684 mg mefloquine base (=750 mg salt) p.o. as the initial dose, followed by 456 mg base (=500 mg salt) p.o. given 6–12 hr after the initial dose for a total dose of 1250 mg salt, corresponding to a dose of 25 mg/kg salt within a 12-hr period for a 50-kg person (CDC, 2013). The recommended dose for malaria prophylaxis in the Roche Lariam label is one 250 mg Lariam tablet (approximately 5 mg/kg for a 50-kg person) once weekly.

In the third edition of the Guidelines for the Treatment of Malaria (WHO, 2015a), mefloquine is recommended for use for the treatment of malaria when administered as part of a combination product with artesunate. Adult tablets contain 100 mg artesunate and 220 mg mefloquine hydrochloride (equivalent to 200 mg mefloquine base). The target doses (ranges) are 4 (2–10) mg/kg body weight per day artesunate and 8.3 (5–11) mg/kg body weight per day mefloquine, given once a day for 3 days. For adults weighing more than 30 kg, the dose is 200 mg artesunate + 440 mg mefloquine hydrochloride daily for 3 days which, for a 50-kg person, corresponds to 4 mg/kg/day artesunate and 8.8 mg/kg/day mefloquine hydrochloride.

EXPERIENCE IN PREGNANT WOMEN IN THE FIRST TRIMESTER

Two prospective studies reported high rates of abortions with mefloquine exposure in pregnancy. One study reported on US Army servicewomen who took 250 mg mefloquine weekly for malaria prophylaxis (Smoak et al., 1997). There were 12 spontaneous, first trimester abortions among 36 pregnant women who did not have elective abortions. Among these 12 women who aborted, the abortions occurred at a gestational age of 6 to 12 weeks after a total of 2 to 12 doses. This incidence (33%) was higher than the cited background rate of 7 to 12% (Kline et al., 1990; Phillips-Howard and Wood, 1996).

In the second study (Phillips-Howard et al., 1998), data were collected from surveys of women traveling between Kenya and Europe between 1987 and 1992 and from the Roche database. In the traveler cohort, there were 99 exposures to mefloquine in the first trimester for the purpose of malaria prevention, nine spontaneous abortions, and no infants with congenital anomalies. For the Roche database, results with mefloquine were compared with those with sulfadoxine + pyrimethamine (SP) for women who had been exposed in the first trimester primarily for the purpose of prevention of malaria. Among 446 women who were exposed to mefloquine, 115 (26%) had therapeutically induced abortions undertaken due to a perceived risk to the fetus and 30 (9.1% of 331) had spontaneous abortions which both resulted in a statistically significantly increased relative risk compared with SP for which there were 165 women exposed to SP, 12 (7% of 165) of whom had therapeutically induced abortions and 4 (2.6% of 153) had spontaneous abortions. The group size for SP was relatively small, and the rate of spontaneous abortions seen with mefloquine (9.1%) was comparable to referenced background rates (7% to 11%; Heinonen et al., 1977; Kline et al., 1990). In the mefloquine group, there were 16 infants with congenital anomalies among 301 deliveries (5.3%), which was considered to be comparable to the provided background rate of 4.6% for whites delivering at 12 hospitals in 10 major American cities from 1959 to 1965 (Heinonen et al., 1977).

A subsequent postmarketing surveillance study based on the Roche database included primarily European travelers with a cutoff date of September 10, 1996 (Vanhauwere et al., 1998). Among 646 cases for which treatment with mefloquine (primarily for the prevention of malaria) before and/or during pregnancy had been reported prospectively and there was a subsequent delivery and knowledge of the pregnancy outcome, there were 26 infants (4.0%) with congenital malformations which was not considered to be different from that in the general population (approximately 2.5%). Among these 646 cases, exposure to mefloquine occurred during the first trimester in 476 cases and the birth prevalence of congenital malformations in this subgroup was 5.0% (24 of 476).

The Roche postmarketing surveillance database was subsequently updated and evaluated again with a cutoff date of October 26, 2010 (Schlagenhauf et al., 2012a,b). Among 978 cases for which exposure to mefloquine before and/or during pregnancy had been reported prospectively and there was a subsequent delivery and knowledge of the pregnancy outcome, there were 43 infants (4.4%) with congenital malformations which was considered to be comparable to the background rates in the general population based on various birth defect databases. Among these 978 cases, exposure to mefloquine occurred during the first trimester in 717 cases and the birth prevalence of congenital malformations in this subgroup was 5.3% (38 of 717).
A retrospective study in 3587 Karen malaria-infected women living along the western border of Thailand evaluated the effect of mefloquine on the outcome of pregnancy (Nosten et al., 1999). Three groups had been exposed during pregnancy or during the 3 months before conception to 25 mg/kg mefloquine (alone or with other antimalarials), quinine, or other antimalarials. The fourth group consisted of pregnant women not infected with malaria and not treated with an antimalarial. The mefloquine group was not different from the other three groups in regard to abortion, low birth weight, neurological retardation, or congenital malformations. However, women who received mefloquine treatment had a significantly greater risk of stillbirth (9 stillbirths among 186 deliveries = 4.8%) compared with each of the other three groups (1.4% to 1.8%). The incidences of stillbirths by stage of pregnancy when exposure occurred were 2.6% (2 of 76) when treated from 3 months before conception to conception, 6.0% (3 of 50) when treated from conception to 4 months of pregnancy and 6.7% (4 of 60) when treated from 4 months of pregnancy through the end of pregnancy. There were six infants with congenital anomalies among 200 women (3%) exposed to mefloquine during pregnancy including 47 women exposed in the first trimester and none of these congenital anomalies was attributed to treatment with mefloquine.

The authors of the previous study referred to an earlier study of theirs in the same community of Karen women (Nosten et al., 1994). The earlier study was a double-blind, placebo-controlled trial of mefloquine prophylaxis in the second half of pregnancy (>20 weeks of gestation) in which there was a higher rate of stillbirths among mefloquine recipients (11 of 159 = 6.9%) compared with placebo (4 of 152 = 2.6%), which was not statistically significant (relative risk = 2.63; 95% CI = 0.86 to 8.08; p = 0.13). The authors believed that the two studies together supported the interpretation that there was a causal link between mefloquine exposure and stillbirth in that community.

Among the articles that met the inclusion criteria for a February, 2014, review (Gonzalez et al., 2014a) were the six articles discussed in the preceding paragraphs and seven articles that found no adverse effects of mefloquine treatment during pregnancy on the rates of abortions, stillbirths, and/or congenital anomalies. In the five of these seven negative studies in which mefloquine was used for the treatment of malaria, there was a total of 7 pregnant women exposed during the first trimester (~10 mg/kg twice, 6 hr apart; Harinasuta et al., 1990), 309 pregnant women exposed to mefloquine alone during the second and third trimesters (Harinasuta et al., 1990; McReady et al., 1998; Adam et al., 2004b), and 88 exposed during the second and third trimesters to the combination of mefloquine with artemether (Sowunmi et al., 1998) or artesunate (McReady et al., 2000).

In the other two articles, mefloquine was used for malaria prevention. In one of these two studies, there were 932 exposures during pregnancy to an initial dose of ~15 mg/kg mefloquine followed by ~5 mg/kg weekly until delivery and included 14 exposures in the first trimester (Steketee et al., 1996). In the other study, 781 women were enrolled between Weeks 16 and 28 of gestation and received two treatments with 15 mg/kg mefloquine at the mean gestational age of 24 and 33 weeks (i.e., there were no exposures during the first trimester). The overall conclusion of this review was that “Based on the evidence reviewed, it can be concluded that mefloquine recipients did not have an increased risk of adverse pregnancy outcomes, including those in the first trimester of gestation.”

Two multicenter randomized controlled trials reported in 2014 found no adverse effects of preventive mefloquine treatment during pregnancy on the rates of abortions, stillbirths, and/or congenital anomalies. One study was conducted in Benin, Gabon, Mozambique, and Tanzania and compared three groups of HIV-negative pregnant women treated with a single 15 mg/kg dose of mefloquine (1579 participants), a 15 mg/kg dose of mefloquine split over two consecutive days (1590 participants), or a single dose of SP (Gonzalez et al., 2014b). There was a total of 266 participants treated with mefloquine in the first trimester. The second study was conducted in Kenya, Mozambique, and Tanzania and compared groups of HIV-positive pregnant women treated with three doses at least 1 month apart of either 15 mg/kg mefloquine or placebo (Gonzalez et al., 2014c). Treatment started in the first trimester for 62 participants in the mefloquine group and 70 participants in the placebo control group.

In an observational study that prospectively followed first trimester pregnancies in women at the Thai-Myanmar border, 25 women with malaria were treated with mefloquine alone and only 2 (8%) miscarried, which was not different from reference groups (p = 0.41; Moore et al., 2016). At maternally toxic doses, mefloquine caused embryo death in rats, rabbits, and mice and malformations in rats (e.g., hydrocephaly) and mice (cleft palate). In addition, mefloquine was a selective developmental toxicant in rabbits and mice. In dose groups treated sequentially with 80 mg/kg on GD 6 followed by 40 mg/kg on GD 9, 12, and 15, there were treatment-related embryo death in two separate rabbit studies but maternal toxicity in only one of the two studies. Treatment of mice with 150 mg/kg/day mefloquine on GD 9 to 11 caused cleft palate but no maternal effects.

The ability to compare kinetic data for mefloquine at a dNOEL in a studied animal species to that seen with a therapeutic dosing regimen in people is very limited. In one rat study, a single dose of 45 mg/kg or 187 mg/kg
mefloquine was selected to match the human exposure to mefloquine following a prophylactic or therapeutic dose, respectively, although the kinetic data in both rats and humans were based on a single time point. Also, the kinetic data in rats were from sexually immature female rats of a different strain than that used in the developmental toxicity study. Neither of these doses was a clear dNOEL in rats.

The HEDs at the dNOELs in rats were approximately 3 to 4 mg/kg/day with treatment throughout organogenesis and 5 mg/kg/day with treatment on GD 9 to 11. The HED at the dNOEL was ~2 mg/kg/day in both rabbits and mice. These values extend up to the prophylactic dose range (~5 to 15 mg/kg/day) but are lower than the therapeutic dose of mefloquine for treatment of malaria either when administered alone (25 mg/kg) or when taken as part of a combination with artemunate (approximately 9 mg/kg/day). Thus, the HED ratios for treatment of malaria based on these animal studies are less than 1.

Selective developmental toxicity was observed at HEDs considered to be ~5 mg/kg/day in rabbits (the effect was embryo deaths) and 12 mg/kg/day in mice (the effect was cleft palate) which are in the therapeutic range.

The coadministration of mefloquine at 30 and 80 mg/kg/day had an ameliorative effect on the developmentally toxic effects of artemunate seen when administered to rats at 15 and 40 mg/kg/day, respectively.

Thus, the animal data warned of possibly serious effects of mefloquine on pregnancy (embryo death and malformation) at relatively low doses. However, there have now been more than 1200 recorded exposures to mefloquine in the first trimester for which the pregnancy outcome is known. Seventy-nine of these exposures occurred in women treated for malaria with the therapeutic dose (~25 mg/kg administered within 24 hr) and more than 1100 exposures occurred in women administered prophylactic doses (~5 to ~15 mg/kg sometimes administered intermittently), most of whom were not infected with malaria. There may be some concerns in some populations but most of the data suggest that clinically used doses of mefloquine do not have markedly adverse effects on pregnancy in any trimester despite the results of the animal studies.

In 2011, the CDC changed their recommendation regarding the use of mefloquine to say that they recommend mefloquine for pregnant women in all trimesters both as a malaria treatment option and as an option to prevent malaria infection (CDC, 2011, 2013). The change in recommendation was based on a recent FDA recategorization of mefloquine from a pregnancy category C drug to category B, which was considered to be a lower risk category.

**Piperazine**

**STUDIES IN PREGNANT ANIMALS**

A complete package of ICH-compliant developmental toxicity studies in rats and rabbits was reported on in Longo et al., 2012, in which piperazine doses referred to mg/kg/day of piperazine phosphate (factor 1.18). In addition, Batty et al. (2010), conducted a reproductive toxicity study in mice. These studies are described immediately below.

**Rat studies.** Design features of five studies in pregnant rats (Studies A, B, C, F, and G) are shown in Table 15 (Longo et al., 2012). A DRF study (Study A) was conducted in pregnant rats with some groups dosed on GD 7 through 17 and terminated at cesarean section on GD 20 and with other groups that were allowed to deliver and were dosed through lactation Day 7. A standard, ICH-compliant definitive EFD study (Study B) was conducted. An additional EFD-like study was conducted subsequently (Study C) except with a single dose level (80 mg/kg/day), a smaller group size (12 pregnant females), a different treatment period (GD 6 to 21), and a different day of cesarean section (GD 21).

An additional DRF study (Study F) was conducted in pregnant rats with treatment from GD 19 to the first week of lactation. Based on the observation of dystocia resulting in perinatal mortality in Studies A and F, the treatment period for the 20 and 80 mg/kg/day piperazine groups in the definitive pre- and postnatal development (PPN) study (Study G) was modified by omitting treatment during gestation after GD 17 in an effort to circumvent the dystocia issue and allow live litters to be delivered for postnatal evaluation (see Table 15).

In the definitive EFD study (Study B; 22 to 24 pregnant rats per group; Table 15), wavy ribs were induced in rats at doses of 40 mg/kg/day (incidence of 11 fetuses from 7 of 24 litters) and 80 mg/kg/day (18 fetuses from 7 of 23 litters) compared with none in the control (23 litters). Effects on maternal food consumption and body weight gain were also seen at those doses. Wavy ribs can result from myometrial contraction (Nakatsuka, 1988) and maternal metabolic alkalosis (Nakatsuka et al., 1993) and are commonly observed in association with maternal and fetal toxicity (Carney and Kimmel, 2007). A low incidence of wavy ribs was also observed in the 20 mg/kg/day group (2 fetuses from 2 of litters) compared with none in the control. In the subsequent smaller study (Study C; 12 pregnant females/group) with treatment at 80 mg/kg/day from GD 6 to 21, there was no difference in the incidences of wavy ribs between the 80 mg/kg/day group (4 fetuses from 1 of 12 litters) and control (6 fetuses from 1 of 12 litters).

On lactation Day 0 in the PPN toxicity study (Study G), the group treated with 80 mg/kg/day piperazine on GD 6 through 17 had 3 dead pups from one litter with wavy ribs (among approximately 27 dead pups examined) compared with 0 among approximately 6 dead pups examined in the control group. There were also treatment-related 5% decreases in fetal weight at 80 mg/kg/day in both Studies B and C. Given the control incidence of 6 fetuses...
| Termination                  | Study designation | Study type/treatment period | Dose (mg/kg/day) | Group size | Maternal findings | Fetal weight | Wavy ribs (fetuses/litters) | Comments |
|-----------------------------|-------------------|-----------------------------|------------------|------------|------------------|--------------|-----------------------------|----------|
| C-Section on GD 20          | A                 | DRF (GD 6-17)              | 40               | 5^a        | mNOEL            |              |                | Reduced | - | |
|                             |                   |                             | 80               | 5^a        | Reduced          | -            |                | - | - | |
|                             |                   |                             | 120              | 5^a        | -                | -            |                | - | - | |
| B                           | Definitive EFD    | (GD 6-17)                  | 20               | 22         | mNOEL            | -            | 2/2 (0 in control) | dNOEL |
|                             |                   |                             | 40               | 24         | Reduced food consumption and body weight gain; enlarged spleen | - | 11/7 |
|                             |                   |                             | 80               | 23         | 5% Decrease      | 18/7         |                | - | - | |
| C                           | Additional EFD    | Study (GD 6-21)            | 80               | 12         | As seen previously | 5% Decrease | 4/1 (6/1 in control) | - | - | |
| Delivery, postnatal evaluation | A                     | DRF (GD 6 to LD 7)         | 40, 80, 120      | 5^a        | Prolonged gestation and parturition | Increased | perinatal pup mortality | - | - | |
with wavy ribs from 1 of 12 litters in Study C, the two fetuses from 2 of 22 litters at 20 mg/kg/day in the definitive EFD study (Study B) are not considered treatment-related and the dNOEL is considered to be 20 mg/kg/day. In Longo et al. (2012), the piperaquine-related incidences of wavy ribs at 40 and 80 mg/kg/day and the 5% decreases in fetal weight at 80 mg/kg/day were not considered to be adverse and 80 mg/kg/day was indicated as the dNOAEL.

In the groups in Study A that were treated on GD 6 through lactation Day 7 and allowed to deliver offspring, there was prolongation of gestation and parturition at all doses (40, 80, and 120 mg/kg/day) resulting in perinatal mortality in some animals. In Study F, starting treatment late in gestation (GD 19) still resulted in prolonged duration of gestation and parturition and perinatal mortality at doses of 60 mg/kg/day and greater. The elimination of treatment during gestation after GD 17 in the 20 mg/kg/day group was successful in preventing perinatal mortality associated with dystocia but not entirely successful in the 80 mg/kg/day group. Thus, the NOEL for prolonged gestation and parturition resulting in perinatal mortality was 5 mg/kg/day with dosing through the end of gestation and 20 mg/kg/day for dosing on GD 6 through 17. In Study G, there was perinatal mortality observed in association with dystocia at 80 mg/kg/day but no other effects of treatment on the survival, growth or development of the F1 generation.

Rabbit studies. A DRF study (Study D) and an ICH-compliant definitive EFD study (Study E) were conducted in pregnant rabbits with dosing on GD 6 to 20 (Longo et al., 2012). Design features of these studies are shown in Table 16.

There were no developmental effects at any dose level (20, 40, and 80 mg/kg/day) in the rabbit EFD study (Study E) so the dNOEL in rabbits was 80 mg/kg/day. Plasma concentrations of piperaquine were evaluated following the first and last doses in the definitive EFD study (Study E). No piperaquine was detected in the plasma at 20 and 40 mg/kg/day. On GD 20 in Study E, the Cmax and AUC0-last in the 80 mg/kg/day group (single animal) were 102 ng/ml and 2260 ng.h/ml. No data were provided for exposure to piperaquine following the first dose at 80 mg/kg/day. Selected toxicokinetic results are shown in Table 17.

Mouse study. Groups of 20 to 23 pregnant mice were treated with 30, 100, or 300 mg/kg/day piperaquine on GD 14 to 18 and then allowed to deliver (Batty et al., 2010). The F1 offspring were reared to sexual maturity and mated to produce an F2 generation. Subgroups of both F1 and F2 mice were evaluated at 4 weeks of age for biochemical and hematological indices and liver and kidney histopathology. No significant adverse effects of piperaquine treatment were detected.
THERAPEUTIC DOSING REGIMEN
Each tablet of the piperaquine+DHA combination (Eurartesim®) contains 320 mg piperaquine tetraphosphate-tetrahydrate and 40 mg DHA. According to the EMA’s Public Assessment Report (EPAR) for Eurartesim® (CHMP, 2011), for body weights in the range of 36 to 75 kg (average 55.5 kg), the recommended dose for malaria is 3 tablets once daily for 3 days for a total dose of 960 mg piperaquine tetraphosphate and 120 mg DHA per day and, for a 50-kg human, an approximate dose of piperaquine tetraphosphate of 19.2 mg/kg/day. In adults treated for 3 days with a therapeutic dose, the AUC0-24h for piperaquine following the third dose was 15.4 l g.h/ml (Longo et al., 2012).

EXPERIENCE IN PREGNANT WOMEN IN THE FIRST TRIMESTER
In one study (Poespoprodjo et al., 2014), five of eight women treated for malaria with oral DHA+piperaquine in the first trimester miscarried compared with 1 of 38 women treated with quinine (p < 0.001). According to hospital policy, quinine and clindamycin were the treatment of choice in the first trimester. It is not clear how the patients to be treated with DHA+piperaquine in the first trimester were selected.

ASSESSMENT
There are only limited data regarding the treatment of women in the first trimester with piperaquine or the combination of piperaquine+DHA.

There were no unusual effects of piperaquine on development in rats or rabbits. The developmental effects that occurred did so in only one species (rat) and only at doses that also caused maternal effects so there was no selective developmental toxicity. Piperaquine did not cause any malformations even at maternally toxic doses. Perinatal mortality was observed in rats following treatment throughout organogenesis but was associated with and likely secondary to dystocia. The other developmental effects observed in rats (wavy rib and decreased fetal weight on GD 20) are typically reversible and did not result in adverse postnatal effects in the PPN study.

The dNOELs and AUC0-24h values for the definitive rat and rabbit studies with dosing just during organogenesis are shown in Table 17.

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**Table 16. Piperaquine: Summary of Results in Pregnant Rabbit Studies**

| Study designation | Study type | Dose - mg/kg/day | Group size | Maternal findings | Adverse effects | Ext/visc malfs |
|-------------------|------------|-----------------|------------|------------------|----------------|--------------|
| D                 | DRF        | 40              | 6<sup>a</sup> | Slight reduction in body weight gain and food consumption, liver changes (yellowish and firm) | None | - |
|                   |            | 80              | 4          |                  | None           |              |
|                   |            | 120             | 6<sup>a</sup> | Death, abortion, body weight loss, reduced food and water consumption, liver changes | NR | NR |
| E                 | EFD        | 20              | 18         | mNOEL            | None           | None         |
|                   |            | 40              | 17         | 2 females with hepatobiliary changes | None, dNOEL   |              |
|                   |            | 80              | 17         | Toxicity limited to 4 females included death, abortion, body weight loss, reduced food and water consumption, hepatobiliary changes; one other female with hepatobiliary changes | None, dNOEL   |              |

From Longo et al., 2012.

<sup>a</sup>Number assigned to group.

DRF, dose-range-finding study; EFD, embryo-fetal development study; NR, not reported; dNOEL, developmental no effect level.
A conservative approach is to select the dose of 20 mg/kg/day in pregnant rats as the dNOEL (based on the observation of wavy ribs at 40 mg/kg/day) for comparison to exposure at the therapeutic dose in humans. The AUC₀₋₂₄h at that dose ranged from 1.8 μg.h/ml (on GD 6) to 4.3 μg.h/ml (on GD 17) compared with an AUC₀₋₂₄h of 15.4 μg.h/ml at the therapeutic dose in humans. The corresponding rat HED at the dNOEL was 3.2 mg/kg/day compared with the therapeutic dose of 16 to 19 mg/kg/day in humans. Thus, the exposure ratios for piperaquine in rats were less than 1. They were also low in rabbits for which the dNOEL was the highest dose tested, 80 mg/kg/day compared with an AUC₀₋₂₄h of 80a 12.9 μg.h/ml at the therapeutic dose in humans. The corresponding rat HED at the dNOEL was 25.8 mg/kg/day and the AUC ratio was 0.15.

Overall, there were no unusual findings related to the administration of piperaquine during organogenesis in rats and rabbits. The estimated exposure ratios for rats and rabbits following treatment throughout organogenesis were ≤1.4.

### Sulfadoxine and Pyrimethamine

**ROLE OF FOLATE IN EMBRYOGENESIS AND IN THE MECHANISM OF ACTION OF SULFADOXINE AND PYRIMETHAMINE**

Both sulfadoxine and pyrimethamine are inhibitors of the biosynthesis of tetrahydrofolate (THF) which, because of its role in one carbon metabolism, is needed by dividing cells to make nucleotides and amino acids (Hitchings and Burchall, 1965; Pietrzik et al., 2010). Sulfadoxine is one of a group of sulfonamides that inhibits dihydropteroate synthetase (DHPS), which converts dihydropteroate + p-aminoenzoic acid to dihydropteroic acid, which is subsequently converted to dihydrofolic acid (Hitchings, 1973). Pyrimethamine is an inhibitor of dihydrofolate reductase (DHFR) that converts dihydrofolic acid to THF (Ferone et al., 1969).

Folic acid (vitamin B₉) does not occur naturally but is used in fortified foods or in dietary supplements as a source of "folate." It must first be reduced to THF to be biologically active (Goh and Koren, 2008; Pietrzik et al., 2010). Folic acid in the intestines is absorbed into the intestinal mucosal cells where it is reduced to first dihydrofolate and then THF by DHFR and then converted to 5-methyl-THF (levomefollic acid) before it is released into the blood (WHO, 2002). 5-Methyl-THF is the only folate species normally found in the circulation (Pietrzik et al., 2010). It has been observed that 5-methyl-THF reactivates mammalian DHFR in the presence of DHFR inhibitors (Goldman and Matherly, 1987) allowing some purine/pyrimidine synthesis to proceed even in the presence of a DHFR inhibitor.

Folic acid deficiency in humans has been associated with increased incidences of malformations (Hibbard and Smithells, 1965) and periconceptional supplementation with folic acid in humans has been shown to reduce the incidence of neural tube defects and other types of malformations (Lumley et al., 2001; De-Regil et al., 2010; Yang et al., 2016). Accordingly, folic acid supplementation has been recommended by various public health groups (Green, 2002). A commonly recommended daily dose is 0.4 mg/day (Institute of Medicine, 1998; Green, 2002; Goh and Koren, 2008; WHO, 2015b). However, it has been reported that even a dose of 0.8 to 1.1 mg/day provides only partial protection against neural tube defects (Wald et al., 1998; Goh and Koren, 2008). For women taking intermittent preventative treatment in pregnancy with SP (IPTp-SP), there is a concern that the use of folic acid during IPTp-SP could diminish the antimalarial activity of SP (Peters et al., 2007). It has been shown that IPTp-SP remains effective with a daily dose of 0.4 mg folic acid and Peters et al. (2007), suggested that "0.4 mg of folic acid supplementation per day [the current WHO recommended dose] may provide an optimal balance to prevent neural tube defects and maintain the effectiveness of IPTp-SP."

Antifolate agents are selectively toxic to rapidly dividing cells such as bacteria, malarial parasites, cancer cells and embryonic cells. Some antifolate agents including

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**TABLE 17. Piperaquine: Exposure at dNOEL in Animal Studies**

| Species | Study      | dNOEL (mg/kg/day) | HED (mg/kg/day) | AUC₀₋₂₄h (μg.h/ml) |
|---------|------------|-------------------|-----------------|-------------------|
| Rat     | EFD        | 20                | 3.2             | Day 6 Day 17      |
|         |            |                   |                 | 1.8 4.3           |
|         |            | 80a               | 12.9            | 9.3 10.8          |
|         | PPN        | 20                | 3.2             | -                 |
|         |            | 80                | 25.8            | 2.3               |

From Longo et al., 2012. Clinical dose: 16 to 19 mg/kg/day. Clinical exposure: AUC₀₋₂₄h for piperaquine following the third dose (~18.3 mg/kg/day) was 15.4 μg.h/ml.

*Developmental no adverse effect level as interpreted by Longo et al., 2012.

EFD, embryo-fetal development study; dNOEL, developmental no effect level; HED, human equivalent dose; PPN, pre- and postnatal development study.
Pyrimethamine are known animal teratogens. Sulfonamides inhibit DHPS and sulfonamides other than sulfadoxine have caused cleft palate and other malformations in rats and mice (Peters et al., 2007). The DHFR-inhibiting anticancer agent methotrexate caused hydrocephalus, cleft palate, and limb defects in rabbits (Jordan et al., 1977). The mechanism for the teratogenic consequences (in particular, neural tube defects) induced in folate deficiency models has been extensively studied, primarily in mice (Hansen, 1997; Antony and Hansen, 2000; Hansen et al., 2003; Ferguson et al., 2005; Xiao et al., 2005; Tang et al., 2011).

Discussed below in the section on animal studies of pyrimethamine are studies that have examined the effects of folic acid and folinic acid (5-formyl-THF; leucovorin) on the teratogenicity of pyrimethamine. Folinic acid is readily converted to tetrahydrofolate and other active forms without the action of DHFR.

There is evidence of increased risk for malformed human offspring resulting from the administration during the first trimester of the following: specific sulfonamides, such as sulfamethoxypyridazine and sulfathiourea (Czeizel et al., 2001); specific DHFR inhibitors, such as aminopterin (Thiersch, 1952), triamterene, sulfasalazine, and trimethoprim (Hernandez-Diaz et al., 2000, 2001); specific combinations of a DHFR inhibitor and a sulfonamide, such as trimethoprim-sulfamethoxazole and trimethoprim-sulfamethazine (Czeizel et al., 2001); and lumped groupings of antifolate agents (Nelson and Forfar, 1971; Hernandez-Diaz et al., 2000, 2001; Czeizel et al., 2004).

The types of malformations associated with antifolate treatment in humans include neural tube defects, oral clefts, and malformations of the limb, cardiovascular system, and urinary tract. Pyrimethamine is a DHFR inhibitor but it has two features that make it selectively toxic to malarial parasites compared with the human host. First, pyrimethamine is 1000-fold more active against the plasmodial DHFR than against the mammalian enzyme (Ferone et al., 1969). Second, further selectivity of DHFR inhibitors for the plasmodial enzyme is provided by the DHFR inhibitor-induced upregulation of the synthesis of the mammalian DHFR but not the plasmodial enzyme (Zhang and Rathod, 2002).

STUDIES IN PREGNANT ANIMALS

**Pyrimethamine.** The results of oral studies of pyrimethamine in pregnant rats are summarized in Table 18. A single oral dose of ~10 mg/kg on GD 9 and doses of ~2 mg/kg/day on GD 9 to 11 and GD 11 to 15 caused malformations including cleft palate, micrognathia, and limb defects (Dyban et al., 1965). Cleft palate and mandibular malformations were also seen with 3.6 mg/kg/day in the diet on GD 11 to 15 (Kudo et al., 1988, 1993) and with 2.7 mg/kg/day p.o. on GD 7 to 17 (Chung et al., 1993). Increased resorptions were seen with 0.3 mg/kg/day pyrimethamine (referred to as 2, 4-diamino-5-p-chlorophenyl-6-ethylpyrimidine and 50-63) on GD 7 to 16 (Thiersch, 1954), ~4 to 8 mg/kg on GD 10 and ~18 mg/kg on GD 12 (Sullivan and Takacs, 1971), and with 2.7 mg/kg/day on GD 7 to 17 (Chung et al., 1993). The reporting of maternal effects was incomplete but an effect on maternal body weight gain observed at 2.7 mg/kg/day in Chung et al. (1993), was likely related to the 38% postimplantation loss observed in that group. Thus, it is likely that pyrimethamine was a selective developmental toxicant in rats.

Exposure to nonpregnant rats was measured following a single oral gavage dose of 3.6 mg/kg pyrimethamine (Kudo et al., 1993). It was estimated that the Cmax was approximately 0.030 µg/ml and the AUC0-24h approximately 0.34 µg.h/ml.

The results of oral studies of pyrimethamine in pregnant mice, hamsters and minipigs are summarized in Table 19. In minipigs, there were increased incidences of newborns with cleft palate, micrognathia, digit defects, and other malformations when administered 3.6 mg/kg/day pyrimethamine in the diet on GD 11 to 35, GD 11 to 22, and GD 11 to 16 and increased digit defects were seen with treatment of 3.6 mg/kg/day in the diet on GD 17 to 22 (Misawa et al., 1982; Yamamoto et al., 1984; Hayama and Kokue, 1985). Increased resorptions were seen at 50 mg/kg/day (in diet) in mice and at ~230 mg/kg/day p.o. in hamsters (Kudo et al., 1993; Sullivan and Takacs, 1971). There was no reporting of maternal effects in these species.

The results of studies which examined the effects of folic acid and folic acid on pyrimethamine-induced embryotoxicity are shown in Table 20. The coadministration of folic acid reduced or prevented the embryotoxicity including teratogenic effects of pyrimethamine in rats (Sullivan and Takacs, 1971; Morse et al., 1976; Kudo et al., 1988, 1993; Chung et al., 1993) and mice (Kudo et al., 1993). Also, the coadministration of folic acid intraperitoneally reduced the teratogenicity of pyrimethamine in rats (Kudo et al., 1993). Interestingly, cotreatment with oral folic acid, i.e., in the diet or by gavage, resulted in the potentiation of the teratogenicity of pyrimethamine in both rats (Kudo et al., 1988, 1993; Hayama et al., 1991) and mice (Kudo et al., 1993). Kudo et al. (1993) provided a possible explanation for this phenomenon. These authors observed that there was an inverse correlation between the plasma levels of 5-methyl-THF and pyrimethamine-induced embryotoxicity. They also observed that oral folic acid led to decreased plasma levels of 5-methyl-THF. It was suggested that oral folic acid potentiates the embryotoxicity of pyrimethamine by inhibiting the absorption of 5-methyl-THF (from food), a phenomenon that had been reported previously (Selhub et al., 1984).

**Sulfadoxine.** The results of oral studies of sulfadoxine in pregnant rats and rabbits are summarized in Table 21.
| Strain     | Treatment period | Dose      | Litters evaluated | Maternal findings | Developmental findings | Reference               |
|------------|------------------|-----------|-------------------|-------------------|------------------------|-------------------------|
| Long-Evans | GD 7-16          | 0.3 mg/kg/day | 14                | 21.7% weight gain from GD 1 to 21 compared to 36.1% in control. | Control group size: 37; increased resorptions (6%) at 0.3 mg/kg/day compared to control (0.9%) and 15% decrease in mean fetal weight compared to control | Thiersch, 1954          |
| Wistar     | GD 10            | 1 or 2 mg (~4 to 8 mg/kg) | 9                | Not reported | 7% resorptions compared to 1% in the control group (N = 15) | Sullivan and Takacs, 1971 |
| Wistar     | GD 10            | 5 mg (~19 mg/kg) | 13                | Not reported | 76% resorptions compared to 1% in the control group (N = 15); 4 of 35 fetuses (11%) were abnormal compared to 2.6% of controls | Sullivan and Takacs, 1971 |
| Wistar     | GD 12            | 5 mg (~18 mg/kg) | 11                | Not reported | 31% resorptions compared to 3% in the untreated control group (N = 14); 63 of 91 fetuses (69%) were abnormal compared to 0% of controls | Sullivan and Takacs, 1971 |
| Wistar     | GD 11-15         | 1.6 mg/kg/day (in diet) | 6                | Not reported | No effect on malformation rate (0 of 78 fetuses malformed) | Kudo et al., 1993       |
| Wistar     | GD 11-15         | 3.6 mg/kg/day (in diet) | 6                | Not reported | All 79 fetuses malformed including cleft palate and brachygnathia compared to 0 of 52 fetuses from 4 litters in the control group | Kudo et al., 1993       |
| Wistar     | GD 11-15         | 1.6 mg/kg/day (in diet) | 5                | Not reported | No effects on rates of resorptions and malformations | Kudo et al., 1993       |
| Wistar     | GD 11-15         | 3.6 mg/kg/day (in diet) | 5                | Not reported | 100% of fetuses malformed including cleft palate and brachygnathia; 23% decrease in fetal weight, no effect on resorption rate | Kudo et al., 1993       |
When rats were treated on GD 8 to 14, there were no developmental effects at 267 mg/kg/day (HED = 43 mg/kg/day) but there was a low incidence of cleft palate at 400 mg/kg/day (HED = 65 mg/kg/day; P.J. Fraser, unpublished data, from H.J. Scholer, unpublished data). In a multi-generation study in rats, sulfadoxine was administered in the diet to the F0 (parental) generation animals from 60 days before the first mating of the F0 animals to produce the first (F1a) litters through the weaning of the second (F1b) litters (Bohni et al., 1969; note: sulfadoxine is referred to as sulphormethoxine in this article). There were no developmental effects at either dose (15 and 150 mg/kg/day; HED = 2.4 and 24 mg/kg/day, respectively) in either set of litters (F1a and F1b). Also, there was no discussion of maternal effects. In an oral gavage study in which rabbits were administered sulfadoxine on GD 8 to 16 at 40, 80, and 160 mg/kg/day, there were no effects on resorption rate or fetal weights and no external or skeletal defects (dNOEL = 160 mg/kg/day; HED = 52 mg/kg/day). Again, there was no discussion of maternal effects.

**Sulfadoxine + Pyrimethamine.** The results of studies of the combination of sulfadoxine and pyrimethamine (SP) in pregnant rats and rabbits are summarized in Table 22. In an oral study in pregnant rats with treatment on GD 8 to 16, there were no adverse developmental effects with the combined administration of 16.5 mg/kg/day sulfadoxine and 0.84 mg/kg/day pyrimethamine (P.J. Fraser, unpublished data, from Phillips-Howard and Wood, 1996). With the administration of a much higher dose (204.5 mg/kg/day S + 10.2 mg/kg/day P) on a single day of gestation (GD 11), approximately 50% of the fetuses had cleft palate (P.J. Fraser, unpublished data, from H.J. Scholer, unpublished data). Among groups of rats treated intramuscularly on just 2 or 3 GDs with the same dose level of SP (0.69 mg/kg/day S and 0.034 mg/kg/day P), there was no detected developmental effect when the treatment days were GD 15 and 22 but 100% embryo deaths when the treatment days included either GD 10 or the combination of GD 5 and 12 (Uche-Nwachi, 1998; Uche-Nwachi and Caxton-Martins, 1998). None of these reports included a description of maternal effects.

Sulfadoxine alone had a much higher dNOEL in rats (267 mg/kg/day, P.J. Fraser, unpublished data) than the dose of sulfadoxine (16.5 mg/kg/day, P.J. Fraser, unpublished data) included at the dNOEL for the combination of 20 parts sulfadoxine and 1 part pyrimethamine. In contrast, the dNOEL for pyrimethamine alone when administered to rats, 1.2 mg/kg/day by oral gavage (Chung et al., 1993) and 1.6 mg/kg/day in the diet (Kudo et al., 1993), is similar to the dose of pyrimethamine (0.84 mg/kg/day, P.J. Fraser, unpublished data) included at the dNOEL for SP in rats. However, an even lower dose of pyrimethamine (0.3 mg/kg/day) was teratogenic in an earlier study (Thiersch, 1954). The developmental toxicity of the

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**Table 18. Continued**

| Strain     | Treatment period a | Dose            | Litters evaluated | Maternal findings | Developmental findings |
|------------|--------------------|-----------------|-------------------|-------------------|-----------------------|
| Sprague-Dawley | GD 7 to 17        | 1.2 mg/kg/day   | 10                | None              | No effect on embryo survival or development. |
|            |                    | 2.7 mg/kg/day   | 10                | Weight gain       | Between GD 7 and 12; 71% of fetuses had body weight gain compared to 5% in control group. |
|            |                    |                 |                   | Postimplantation loss | 14 foetal skeletal anomalies compared to 0 control fetuses. |
|            |                    |                 |                   |                   | 37 fetuses had skeletal anomalies compared to none in the control group. |

GD, gestational day.
| Species/Strain       | Treatment period | Dose                  | Litters evaluated | Developmental findings                                                                                                                                                                                                 | Reference                        |
|---------------------|------------------|-----------------------|-------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------|
| Mouse/ICR           | GD 9 to 15       | 50 mg/kg/day (in diet)| 11                | 18% resorptions compared to 1.9% in controls (N = 8 dams); 19% of fetuses with malformations compared to 0.7% in control group                                                                                           | Kudo et al., 1993                |
| Golden Hamster      | GD 9             | 20 mg (~165 mg/kg)    | 5                 | No effect on incidences of resorptions or abnormalities                                                                                                                                                               | Sullivan and Takacs, 1971        |
|                     |                  | 30 mg (~230 mg/kg)    | 3                 | 15 resorptions among 38 implantation sites (40%) compared to 4 of 198 (2%) in lower dose groups (N = 18)                                                                                                               |                                  |
| Minipig, Goettingen | GD 11-35         | 1.8 mg/kg/day (in diet)| 4                 | No malformations among 16 newborns                                                                                                                                                                                   | Misawa et al., 1982              |
|                     |                  | 3.6 mg/kg/day (in diet)| 3                 | 11 of 16 newborns (69%) from 3 litters had cleft palates; other treatment-related malformations included clubfoot, micrognathia, oligodactyly, and polydactyly; among 130 untreated newborns that died prior to weaning, 5 had cleft palate (3.8%) and 1 had agnathia (0.8%) | Yamamoto et al., 1984; Hayama and Kokue, 1985 |
|                     |                  | 3.6 mg/kg/day (in diet)| 5                 | Among 16 live newborns, there were 12 with cleft palate, 5 with clubfoot, 2 with micrognathia and 1 with a digit defect                                                                                           | Yamamoto et al., 1984; Hayama and Kokue, 1985 |
|                     |                  | 3.6 mg/kg/day (in diet)| 6                 | Among 24 live newborns, there were 2 with cleft palate, 1 with clubfoot, 3 with micrognathia and 1 with a digit defect                                                                                           |                                  |
|                     |                  | 3.6 mg/kg/day (in diet)| 4                 | Among 16 live newborns, there were 5 with digit defects                                                                                                                                                             |                                  |
|                     |                  | 3.6 mg/kg/day (in diet)| 4                 | Among 16 live newborns, there was 1 with cleft palate                                                                                                                                                              |                                  |

The day evidence of mating was found or the day of mating = Day 0 of gestation. GD, gestational day.
combination in rats appears to be at least largely due to the pyrimethamine component. It seems likely that the coadministration of sulfadoxine does not potentiate the developmental toxicity of pyrimethamine in rats and may even ameliorate it.

Not enough data are available to make the same comparisons in rabbits. The dNOEL for sulfadoxine administered alone to rabbits (160 mg/kg/day, Bohni et al., 1969) was somewhat lower than the dose of sulfadoxine at the dNOEL for SP (300 mg/kg/day, P.J. Fraser, unpublished data) but no higher doses of sulfadoxine alone were tested. Also, no corresponding information regarding the developmental toxicity of pyrimethamine in rabbits is available.

THERAPEUTIC DOSING REGIMEN
When administered as part of an ACT with artesunate, the recommended dosing regimen for SP is a single dose on Day 1 of at least 25 mg/kg sulfadoxine and 1.25 mg/kg pyrimethamine or, for an adult weighing more than 50 kg, 1500 mg sulfadoxine and 75 mg pyrimethamine (WHO, 2015a). The artesunate component of the ACT is administered once daily for a total of 3 days.

CLINICAL EXPOSURE TO PYRIMETHAMINE
Following a single dose of 1500 mg sulfadoxine and 75 mg pyrimethamine to pregnant women in Papua New Guinea, the median \( C_{\text{max}} \) and AUC\(_{0-\infty}\) values for pyrimethamine were 0.47 \( \mu \text{g/ml} \) and 72.1 \( \mu \text{g.h/ml} \), respectively (Karanageewe et al., 2009).

EXPERIENCE IN PREGNANT WOMEN IN THE FIRST TRIMESTER
The safety in human pregnancy of sulfadoxine, pyrimethamine, the combination of sulfadoxine and pyrimethamine (SP), and the combination of SP with artemisinins has been previously reviewed (Phillips-Howard and Wood, 1996; Nosten et al., 2006; Peters et al., 2007; McGready and Nosten, 2010; Angus, 2014; Tagbor et al., 2014). Key information regarding the safety of SP alone in the first trimester is summarized below.

According to H.J. Scholer (unpublished data as reported in Anonymous, 1983), there was only one malformed infant among those born to 67 women included in Roche files who had taken sulfadoxine + pyrimethamine during the first trimester.

In another study discussed previously in the Mefloquine section, data were collected from surveys of women traveling between Kenya and Europe between 1987 and 1992 and from the Roche database (Phillips-Howard et al., 1998). In the traveler cohort, there with 19 exposures to SP in the first trimester, no spontaneous abortions and no infants with congenital anomalies. In the Roche database, there were 165 women who had been exposed to SP in the first trimester primarily for the purpose of prevention of malaria (some of these cases may have also been included in the report discussed in the preceding paragraph, H.J. Scholer, unpublished data). Among these 165 cases, 12 (7% of 165) had therapeutically induced abortions and 4 (2.6% of 153) had spontaneous abortions. There were 12 infants with congenital anomalies (7.8%) including 5 infants with musculoskeletal malformations (5/153 = 3.3%) but none with cleft palate. The overall incidence of 7.8% is high compared with a typical background malformation rate of 3% in some populations (e.g. CDC, 2008). The background rates provided in Phillips-Howard et al., 1998, were (for whites delivering at 12 hospitals in 10 major American cities from 1959 to 1965; Heinonen et al., 1977)

### TABLE 20. Effects of Folinic Acid and Folic Acid on the Embryotoxicity of Pyrimethamine in Rats and Mice

| Species | Pyrimethamine route | Coadministered compound | Route of folate compound | Effect | Reference |
|---------|---------------------|-------------------------|--------------------------|--------|-----------|
| Rat     | Oral gavage         | Folinic acid            | i.p.                     | Reduction | Sullivan and Takacs, 1971 |
|         | Injected            | Folinic acid            | Injected                 | Prevention | Morse et al., 1976 |
| Diet    | Folic acid          | Diet                    |                           | Potentiation | Kudo et al., 1988 |
|         | Folic acid          | i.p.                    |                           | Prevention | Chung et al., 1993 |
| Diet    | Folic acid          | Diet                    |                           | Potentiation | Hayama et al., 1991 |
| Oral gavage | Folic acid      | Orang gavage               |                           | Potentiation | Chung et al., 1993 |
|         | Folinic acid        | i.p.                    |                           | Prevention | Chung et al., 1993 |
| Diet    | Folic acid          | Diet                    |                           | Potentiation | Kudo et al., 1993 |
|         | Folic acid          | i.p.                    |                           | Reduction  | Chung et al., 1993 |
|         | Folic acid          | Diet                    |                           | Prevention | Chung et al., 1993 |
| Mouse   | Oral gavage         | Folic acid              | Oral gavage               | Potentiation | Chung et al., 1993 |
|         | Folinic acid        | i.p.                    |                           | Reduction  | Chung et al., 1993 |

i.p., intraperitoneal.
4.6% for any malformation and 1.2% for musculoskeletal malformations. However, each of the five musculoskeletal malformations was different and there was no consistent pattern to the anomalies occurring in the SP cohort.

In a study in Zambia, there were 135 women who were treated for malaria in the first trimester with 1500 mg sulfadoxine plus 75 mg pyrimethamine, quinine or SP plus quinine (Manyando et al., 2010). Among these 135 women, at least 120 were treated only with SP, none aborted, there were 3 stillbirths, 28 preterm deliveries, 6 that did not continue on study until delivery, and 121 infants that were evaluated for malformations. The incidences of these findings were similar to an untreated group with the possible exception of preterm delivery for which the incidence in the SP and/or quinine group (28/135 = 20.7%) was slightly higher than that in the untreated group (104/684 = 15.2%). Eight infants (6.6%) in the SP and/or quinine group had malformations, including 5 (4.1%) with umbilical hernia compared with the untreated group in which 27 of 596 (4.5%) had malformations and 20 of 596 (3.4%) had umbilical hernias.

ASSESSMENT

Pyrimethamine caused embryo death and/or malformations in rats, mice, hamsters, and minipigs and was likely a selective developmental toxicant in rats. Sulfadoxine caused cleft palate in rats. The combination of SP caused embryo death and cleft palate in rats and embryo death in rabbits. dNOELS in rats were 1.2 mg/kg/day (HED = 0.19 mg/kg/day) for pyrimethamine, 267 mg/kg/day (HED = 43 mg/kg/day) for sulfadoxine and 16.5 mg/kg/day sulfadoxine and 0.84 mg/kg/day pyrimethamine (HED = 2.6 + 0.14 mg/kg/day, respectively) for SP. dNOELs in rabbits were 160 mg/kg/day (HED = 52 mg/kg/day) for sulfadoxine and 300 mg/kg/day sulfadoxine and 15 mg/kg/day pyrimethamine (HED = 97 + 4.8 mg/kg/day, respectively) for SP. The HED ratios for pyrimethamine (0.15) and the SP combination (~0.1) were low in rats. The HED ratio for sulfadoxine alone in rats (~2) was greater than that for pyrimethamine alone. The HED ratio for sulfadoxine alone in rabbits was also ~2 based on the highest dose tested (160 mg/kg/day) being the dNOEL. It is possible that a higher dose would have been a dNOEL as is suggested by the dNOEL in the rabbit study of the SP combination (300 mg/kg/day sulfadoxine + 15 mg/kg/day pyrimethamine; HED ratio ≈ 4; note: pyrimethamine alone was not tested in pregnant rabbits).

The only comparison of findings in animals to those in the clinic based on kinetics is for pyrimethamine alone in rats. Following a single dose by oral gavage to nonpregnant rats at a dose (3.6 mg/kg/day) approximately three-fold the dNOEL by oral gavage (1.2 mg/kg/day), the Cmax (approximately 0.030 µg/ml) was more than 10-fold lower than that following a therapeutic dose in pregnant women.
| Species | Route | Treatment period | Dose (mg/kg/day) | Litters | Developmental findings | Reference |
|---------|-------|------------------|-----------------|---------|------------------------|-----------|
| Rat     | Oral  | GD 8 to 16       | 16.5 0.84       | Not reported | No adverse developmental effects (dNOEL) | P.J. Fraser, unpublished data, from H.J. Scholer, unpublished data, and Phillips-Howard and Wood, 1996 |
| Rat     | Oral  | GD 11            | 204.5 10.2      | About 50% of fetuses had cleft palate | P.J. Fraser, unpublished data, from H.J. Scholer, unpublished data |
| Rat, Wistar | i.m. | GD 5, 12 and 19<sup>a</sup> | 0.69 0.034 | ≤5 | 100% embryo death | Uche-Nwachi 1998; Uche-Nwachi and Caston-Martins, 1998 |
|         |       | GD 10 and 17<sup>a</sup> | 0.69 0.034 | ≤5 | 100% embryo death | |
|         |       | GD 15 and 22<sup>a</sup> | 0.69 0.034 | ≤5 | Mean litter size = 4.8 compared to overall control mean litter size of 5.6; no malformations noted | |
| Rabbit  | Oral  | GD 8 to 16       | 300 15          | Not reported | No adverse developmental effects (dNOEL) | P.J. Fraser, unpublished data, from H.J. Scholer, unpublished data |
|         |       |                   | 400 20          | Increased resorptions; no treatment-related malformations | |

<sup>a</sup>The day evidence of mating was found = Day 0 of gestation and the day of cesarean section was GD 23.

GD, gestational day; dNOEL, developmental no effect level; i.m., intramuscular.
and the AUC0-24h at that dose in rats (0.34 μg.h/ml) was more than 200-fold lower than that following a therapeutic dose in pregnant women (72.1 μg.h/ml). Thus, the Cmax and AUC ratios for pyrimethamine based on rat studies were much less than one.

Despite the low exposure ratios based on the rat, there is no convincing evidence that SP at therapeutic doses causes an increased risk of abortion, stillbirth, or malformation at therapeutic doses in humans. In Manyando et al., 2010, SP was compared with artemether-lumefantrine and, based on the similar results for the two treatments, it was concluded that “exposure to AL [artemether-lumefantrine] in pregnancy, including first trimester, is not associated with particular safety risks in terms of perinatal mortality, malformations, or developmental impairment.” By extension, a similar statement could be made about SP.

Pyronaridine

STUDIES IN PREGNANT ANIMALS

Ni et al., 1982, reported that the oral administration of 84, 165 or 330 mg/kg/day pyronaridine to rats on GD 7 to 9 caused a dose-related incidence of resorptions at all doses. No malformations were noted.

ICH- and GLP-compliant EFD studies were conducted in rats and rabbits (summarized in Table 23). In a standard EFD study in Sprague-Dawley rats (W.J. Yu, unpublished data), groups of 21 pregnant rats were treated on GD 6 to 15 with doses of 47, 140, and 420 mg/kg/day pyronaridine tetraphosphate and cesarean-sectioned on GD 20. Doses were selected based on the results of a preliminary study in which there was one death at 440 mg/kg/day together with decreases in maternal body weight gain and fetal weight. In the definitive EFD study, maternal toxicity at 420 mg/kg/day consisted of 10 deaths, decreases in food consumption and an 8.4% body weight loss (mean = -23.5 g) between GD 6 and 15 compared with a 17.0% body weight gain (mean = +48.7 g) in the control. Among the survivors on GD 20 in this group, there was a 55% decrease in mean body weight gain between GD 6 and 20 compared with control.

Other maternal effects at 420 mg/kg/day consisted of thymic atrophy and enlarged spleens, adrenal glands, and liver. The sole developmental effect at 420 mg/kg/day was a decrease in mean fetal weight in males (8%) and females (14%) compared with control. At 140 mg/kg/day, maternal effects consisted of mean decreases compared with control in food consumption (e.g., a 28% decrease from GD 6 to 7 and 11% from GD 9 to 10) and body weight gain (e.g., a 17% decrease from GD 6-15 and 12% decrease from GD 6-20). There were also enlarged spleens at 47 and 140 mg/kg/day. There were no developmental effects at 140 mg/kg/day (i.e., the dNOEL). This finding conflicts with that of Ni et al. (1982). The Yu EFD study is considered to be definitive since it had a large group size (N = 21) and was conducted under Good Laboratory Practices whereas the study design and results are not fully presented in Ni et al., 1982.

In a standard EFD study in New Zealand White rabbits (W.J. Yu, unpublished data), groups of 20 to 21 pregnant

**TABLE 23. Pyronaridine: Summary of Results of Definitive EFD Studies in Pregnant Rats and Rabbits**

| Species | Treatment period | Dose (mg/kg/day) | Group size | Maternal findings | Developmental findings |
|---------|-----------------|-----------------|------------|------------------|------------------------|
| Rat     | GD 6-15         | 47              | 21         | Enlarged spleens |                        |
|         |                 | 140             | 21         | Decreased food consumption and body weight gain; enlarged spleens | dNOEL |
|         |                 | 420             | 21         | 10 deaths; decreased food consumption and body weight gain; enlarged spleens, adrenal glands, and liver; thymic atrophy | Decreased fetal weight |
| Rabbit  | GD 6-18         | 13              | 20         | mNOEL            |                        |
|         |                 | 40              | 21         | Decreased food consumption | dNOEL |
|         |                 | 120             | 21         | One death; 8 abortions; decreased food consumption and body weight gain | Increased resorptions and decreased fetal weight |

GD, gestational day; EFD, embryo-fetal development study; dNOEL, developmental no effect level; mNOEL, maternal no effect level.
At that dose, there were decreases in fetal weight in doses to females, the Cmax, time at maximal dose (Tmax), and AUC0-24h values in whole blood. Based on data from a toxicokinetic study in Sprague Dawley rats (L. Fleckenstein, unpublished data), it is estimated that, following single doses to females, the Cmax time at maximal dose (Tmax), and AUC0-24h values in whole blood at 70 mg/kg were 380 ng/ml, 4 hr postdose and 5900 ng.h/ml, respectively, and, at 210 mg/kg, 1130 ng/ml, 4 hr postdose and 12,400 ng.h/ml, respectively. In a subsequent study in which rabbits were treated on GD 6 to 18 with doses of 13, 40, and 120 mg/kg/day pyronaridine tetraphosphate and cesarean-sectioned on GD 28. Doses were selected based on the results of a preliminary study in which doses of greater than 200 mg/kg/day caused maternal death, loss of appetite, diarrhea, loss of fur, loss of body weight, and complete resorption of implanted embryos. In the definitive EFD study, there was marked maternal toxicity at 120 mg/kg/day. One female at that dose had stopped eating by GD 13 and was found dead on GD 19. Among eight females that aborted, seven had periods of eating ≤10 g/day (compared with a control mean of 149 to 172 g/day). As a group, there were 70% and 74% decreases in mean food consumption from GD 15 to 16 and from GD 18 to 19, respectively, compared with control.

Overall, there was a mean body weight loss of 4.5% (mean = -160 g) between GD 6 and 28 at 120 mg/kg/day compared with a mean body weight gain of 10.8% (mean = +408 g) in the control. In the 40 mg/kg/day group, there was a mean body weight loss of 30g between GD 6 and 12 compared with a mean gain in the control of 79 g and also there were 16 to 26% decreases in mean food consumption between GD 12 and 28 compared with control. The mNOEL was 13 mg/kg/day. Developmental effects at 120 mg/kg/day consisted of increased resorptions and decreases in fetal weight. At that dose, there were 3.3 resorptions per litter (resulting from four dams with total litter loss and two other dams with 4 resorptions or dead fetuses per litter) compared with 0.5 per litter in control. All four dams with total litter loss and one of the other two had periods of eating ≤ 10 g/day. Also at that dose, there were decreases in fetal weight in males (12%) and females (20%). The middle dose group, 40 mg/kg/day, was the dNOEL.

Pyronaridine concentrates in erythrocytes (Shao, 1990) and kinetic studies in animals measured the concentration of pyronaridine in whole blood. Based on data from a toxicokinetic study in Sprague Dawley rats (L. Fleckenstein, unpublished data), it is estimated that, following single doses to females, the Cmax time at maximal dose (Tmax), and AUC0-24h values in whole blood at 70 mg/kg were 380 ng/ml, 4 hr postdose and 5900 ng.h/ml, respectively, and, at 210 mg/kg, 1130 ng/ml, 4 hr postdose and 12,400 ng.h/ml, respectively. In a subsequent study in which groups of four pregnant Sprague Dawley rats were administered doses of 47, 140, and 420 mg/kg/day pyronaridine tetraphosphate on GD 6 through 15, concentrations of pyronaridine in maternal whole blood were measured at 3 hr after dosing on GD 6, 10 and 15 (L. Fleckenstein, unpublished data). The group mean concentrations are shown in Table 24. Similarly, groups of four pregnant New Zealand White rabbits were administered doses of 13, 40, and 120 mg/kg/day pyronaridine tetraphosphate on GD 6 through 18 and concentrations of pyronaridine in maternal whole blood were measured at 3 hr after dosing on GD 6, 12, and 18 (L. Fleckenstein, unpublished data). The group mean concentrations are shown in Table 25.

**THERAPEUTIC DOSING REGIMEN**

Pyronaridine is commonly administered as part of a combination product with artesunate (Pyramax). Each Pyramax tablet contains 180 mg pyronaridine tetraphosphate and 60 mg artesunate. According to the EMA’s Summary of Product Characteristics for Pyramax (CHMP, 2016), for body weights in the range of 45 to 65 kg, the recommended dose for malaria is 3 Pyramax tablets once daily for 3 days for a total of 540 mg pyronaridine tetrathosphate and 180 mg artesunate per day. For a 50-kg human, this corresponds to 10.8 mg/kg/day pyronaridine tetraphosphate.

**CLINICAL PHARMACOKINETICS**

The results of clinical pharmacokinetic studies are summarized in Croft et al., 2012. Plasma concentrations of pyronaridine were measured intermittently in a clinical study in which Thai patients with uncomplicated malaria were treated with 12 mg/kg/day pyronaridine tetrathosphate in capsules once daily for 3 days (Ramanathan et al., 2005). The Cmax was 120 ng/ml. The overall AUC starting with the first dose (i.e., AUC0→∞) was 29,400 ng.h/ml. Based on Figure 3 in that article, the mean AUC0→24h during the first 3 days of dosing is estimated to be 1700 ng.h/ml. In another study (Feng et al., 1987), groups of three malaria patients (men and women) were treated with single oral doses of 600 mg pyronaridine contained within either enteric-coated tablets or capsules. In the tablet group, the mean body weight was 55kg so the mean dose was 10.9 mg/kg and the Cmax (mean = 130 ng/ml)
occurred at a mean of 4.7 hr postdose (Tmax). In the capsule group, the mean body weight was 56 kg so the mean dose was 10.7 mg/kg and the Cmax (mean = 255 ng/ml) occurred at a mean of 4.7 hr postdose (Tmax).

EXPERIENCE IN PREGNANT WOMEN IN THE FIRST TRIMESTER
No information was found in the literature regarding the safety of pyronaridine or the combination of pyronaridine and artesunate in human pregnancy.

ASSESSMENT
There were no treatment-induced malformations in the EFD studies in either rats or rabbits. The only developmental effect observed in the rat EFD study consisted of decreases in fetal weight at 420 mg/kg/day which was associated with excessive maternal toxicity that resulted in death for nearly half of the mothers treated. There were no developmental effects at 140 mg/kg/day, a dose at which there were effects on maternal food consumption and body weight gain. The high dose in the rabbit EFD study (120 mg/kg/day) was also excessively toxic with one maternal death, eight abortions, and prolonged anorexia associated with effects on maternal body weight gain. At that dose, there were increased resorptions plus dead fetuses (total = 51) compared with control (total = 19). Forty-two of the 51 dead conceptuses in the 120 mg/kg/day group were in litters that exhibited anorexia. Also at that dose (120 mg/kg/day), there was decreased fetal weight. The marked maternal toxicity at 120 mg/kg/day in the rabbit likely contributed to the observed developmental effects seen at that dose. There were no developmental effects at 40 mg/kg/day in rabbits.

The dNOELs for pyronaridine were 140 mg/kg/day (HED = 22.6 mg/kg/day) in rats and 40 mg/kg/day (HED = 12.9 mg/kg/day) in rabbits compared with the therapeutic dose in humans of approximately 9 to 11 mg/kg/day so the HED ratios were ~2 in rats and ~1 in rabbits. The estimation of exposure ratios based on kinetic parameters is confounded by a few factors and requires unproven assumptions including an estimation of rat and rabbit plasma concentrations from blood concentrations based on the in vitro ratio of blood to plasma for rats and rabbits (approximately 2.25 and 3.15, respectively; Croft et al., 2012). Crude estimates based on these assumptions are Cmax ratios of approximately 3 to 5 and 1 for rats and rabbits, respectively, and an AUC ratio of approximately 5 for rats.

Overall, there were no unusual findings related to the administration of pyronaridine during organogenesis in rats and rabbits. The estimated exposure ratios for rats and rabbits following treatment throughout organogenesis were approximately 1 to 5. There is a paucity of experience with pyronaridine in women in the first trimester.

Discussion
Among the nine non-artemisinin antimalarial drugs reviewed here, seven (quine, clindamycin, lumefantrine, mefloquine, pyrimethamine, sulfadoxine, and pyronaridine) caused embryo deaths and/or malformations in at least one animal species. Six of the nine (quine, clindamycin amodiaquine, mefloquine, piperaquine, and pyrimethamine) had HED ratios of less than 1 in at least one animal species. Mefloquine was a selective developmental toxicant in rabbits and mice for which the primary effects were embryo deaths and cleft palate, respectively, and the HED ratio (when compared with the therapeutic dose of mefloquine when administered with artesunate) was 0.2. Pyrimethamine was likely a selective embryotoxicant in the rabbit for which the HED ratio was 0.15. There is also reason for concern regarding pyrimethamine and sulfadoxine as they are members of classes (dihydrofolate reductase inhibitors and sulfonamides, respectively) that include drugs that are likely human teratogens (see the Sulfadoxine and Pyrimethamine section).

For the combinations of SP and artemether+lumefantrine and three of the other drugs (quine, clindamycin, and mefloquine), there are reports in the literature for 304 to >1100 exposures at therapeutic doses in the first trimester with varying comparisons to reference exposures. These studies have not revealed an increase in malformations or embryo death. The lack of clinical findings with these agents is despite the fact that all of these individual drugs caused embryo deaths and/or malformations in at least one animal species and all except lumefantrine and sulfadoxine had at least one exposure ratio <1. Sulfadoxine had an HED ratio of 2 based on both the rat and rabbit. The only drug with all exposure ratios being >2 was lumefantrine with HED ratios of 2.5 in the rat and 17 in the rabbit and Cmax and AUC ratios in the rabbit of 4.6 and 3.5, respectively.

Before reaching conclusions about the safety of quinine, clindamycin, mefloquine, SP, and artemether+lumefantrine in the first trimester, one should consider the factors discussed below.

One factor is the nature and size of the clinical study. The clinical data presented herein include case reports, retrospective studies, prospective studies with study enrollment before knowing the pregnancy outcome and sometimes also before the administration of the antimalarial, and studies with and without the inclusion of internal comparison groups (i.e., not treated with the test antimalarial). For studies that have not detected drug-induced increased incidences of one or more malformations, estimates can be made of the highest incidence of drug-induced malformations that can be excluded depending on the size of the study and the background rate of malformations.

For example, according to the EMEA Guidelines (EMEA, 2008), "If no increased incidence of malformations is
observed within at least 300 first trimester exposed prospectively collected pregnancies with known pregnancy outcomes, the conclusion might be reached that the medicinal product at hand is not responsible for a 10-fold or more increase of the overall incidence of malformations. Furthermore, “If no increased incidence of malformations is observed within at least 1000 first trimester exposed prospectively collected pregnancies with known pregnancy outcomes, the conclusion might be reached that the medicinal product at hand is not responsible for a twofold or more increase of the overall incidence of malformations.” Presumably, these calculations assumed a background malformation rate of ~3%, which is a fairly standard rate for Western nations (e.g., CDC, 2008).

In the patient populations studied in many of the studies of the safety of antimalarials in the first trimester, the reported background rate of birth defects is less than 3% (perhaps due to underreporting and/or underdetection), which then requires larger test populations to make the same statements regarding risk thresholds of 10-fold and twofold. For example, Dellicour et al., 2017, conducted a meta-analysis of studies that were conducted in Africa and along the Thailand-Myanmar border and estimated that “A sample size calculation suggested that 1180 exposed cases (with a ratio of 1:4 unexposed and background rate of 0.9%) would be enough to detect a doubling of risk of any major congenital anomalies detectable by surface examination.” Furthermore, “To exclude a twofold increase in risk of a specific defect with an estimated background rate of 1/1000, 10,748 first trimester artemisinin treatments and 42,992 untreated pregnancies would be needed.”

Another factor is the role of the sensitive period. Obviously, if a drug can only induce a particular malformation during a short period within the first trimester, then the number of women treated for short periods at any time during the first trimester is going to provide an underestimate of the actual risk resulting from treatment just during the sensitive period. As discussed in the Developmental Toxicity Studies in Animals and Their Interpretation section, the rates of miscarriage and malformations can be studied in humans during specific putative sensitive periods which are estimated based on the results of critical period studies in animals (as was done by Moore et al., 2016; Dellicour et al., 2017). These analyses result in a reduced sample size compared with the entire first trimester but may also result in an increased sensitivity for detecting an increased rate of miscarriages or malformations compared with the entire first trimester.

An additional factor is the extent of the examination of the infants delivered on a study. The detection of congenital anomalies obviously depends on the quality of the examination that is performed which can depend on the equipment that is available. Cardiac malformations are the most characteristic malformation caused by artemisinins. As noted in an article advocating routine neonatal screening using pulse oximetry (Hoffman, 2011), even in modern hospitals, approximately 30% of infants with critical congenital heart disease are not diagnosed before being discharged at 2 days of age. Some minor defects are difficult to detect before 1 month of age and are typically diagnosed by echocardiography when older. In general, the clinical studies described here performed only physical examinations with limited follow-up. As noted in Dellicour et al., 2017, “Furthermore no study [included in the meta-analysis] has been designed to assess cardiovascular defects, which were detected as a potential problem in animal reprotoxicity studies in newborns or any other internal defects due to unavailability of appropriate equipment and expertise in the areas where the burden of malaria in pregnancy is highest.”

Finally, the possible impact of malarial infection on the potential developmental toxicity of antimalarials has not been well studied. Pregnancy outcomes have been recorded for significant numbers of women not known to have malaria who were treated in the first trimester with clindamycin or mefloquine (with no identified adverse consequences) but otherwise a large majority of the women included in the clinical studies described herein were infected with malaria at the time of antimalarial treatment. However, in many areas, pregnant women are treated with antimalarials for fever without the confirmation of malaria. If malaria protects against drug-induced developmental toxicity (as has been hypothesized for artemisinins; Clark, 2012), then the pregnancies of uninfected women may be at greater risk to antimalarial treatment.

Based on the clinical experience described, it cannot be concluded that therapeutic doses of quinine, clindamycin, mefloquine and the combinations of artemether + lumefantrine and SP measurably increase the risk of malformations or embryo death when used to treat malaria in the first trimester. It now seems that the animal studies of these drugs, as conducted, overestimated the risk of developmental toxicity in humans. Whether the findings in animals might eventually be found to correlate with a low incidence of adverse pregnancy findings in humans is not known.

For the other 3 drugs evaluated here (amodiaquine, piperaquine, and pyronaridine), there were few or no reported exposures in women in the first trimester. These drugs were studied in rats and rabbits with dosing throughout organogenesis. None of them caused embryo deaths or malformations (even at maternally toxic doses) in either species with the exception of pyronaridine, which caused embryo deaths in rabbits at a dose (120 mg/kg/day) that was excessively toxic to the mothers as it caused the death of one mother and eight abortions. Thus, none of these drugs was a selective developmental toxicant.

Amodiaquine had estimated AUC and HED ratios of 1.1 and 0.7, respectively, in the rabbit. At a dose in the rat that caused only minor effects, the HED ratio was 0.5 and the estimated AUC ratio at the end of the treatment period was 1.1. Piperaquine had AUC ratios of 0.3 in the rat and...
0.15 in the rabbit and HED ratios of 0.2 based on the rat and 1.4 based on the rabbit. Pyronaridine had HED ratios of 2 based on the rat and 1.2 based on the rabbit. These exposure ratios are low compared with the definition provided by the FDA (2011) that an exposure ratio <10 is low. However, they are not low compared with the exposure ratios for quinine, clindamycin, mefloquine, pyrimethamine, and artemether for which, as discussed above, no adverse effects on pregnancy have been detected in clinical studies involving >304 patients in the first trimester.

The exposure ratios for these three drugs being well below 10 is likely to have been at least partially related to the limitation of the doses studied due to maternal toxicity which, in turn, was related to the duration of dosing. It is likely that higher exposure ratios could be achieved by testing for shorter periods during organogenesis. Treatment for shorter periods would still be relevant because the duration of the therapeutic dosing regimen for each of the corresponding ACTs (amodiaquine + artesunate, dihydroartemisinin + piperaquine, and artesunate + pyronaridine) is only 3 days which is a small fraction of the duration of organogenesis in humans (approximately 52 days). Thus, for amodiaquine, piperaquine and pyronaridine, the testing in animals did not reveal findings of concern and the low exposure ratios were likely at least partially related to the standard practice of dosing throughout organogenesis.

Acknowledgments

The author acknowledges the following foundation and companies for the provision of unpublished study reports and summaries: Medicines for Malaria Venture (pyronaridine ± artesunate), Sanofi (amodiaquine ± artesunate), and Roche (mefloquine ± artesunate and sulfadoxine and pyrimethamine ± artesunate). The author also wishes to acknowledge financial support for this project from the Medicines for Malaria Venture.

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