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## Review of evidence

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# Contents

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# Abbreviations

| | |
|---|---|
| ABT | artemisinin-based treatment |
| ACT | artemisinin-based combination therapy |
| aHR | adjusted hazard ratio |
| AL | artemether–lumefantrine |
| ART | antiretroviral therapy |
| AQ | amodiaquine |
| AS | artesunate |
| AUC | area under the curve (of concentration versus time) |
| CI | confidence interval |
| $C_{max}$ | maximum concentration |
| DHA | dihydroartemisinin |
| dLOEL | developmental low-effect level |
| dNOEL | developmental no-effect level |
| GD | gestation day |
| HED | human equivalent dose |
| MQ | mefloquine |
| non-ABT | non-artemisinin-based treatment |
| PPQ | piperaquine |
| PYR | pyronaridine |
| QNN | quinine |
| SP | sulfadoxine–pyrimethamine |
| TDR | UNICEF–UNDP–World Bank–WHO Special Programme for Research and Training on Tropical Diseases |
| WHO | World Health Organization |

# Definitions

The following definitions *(1, 2, 3)* were used.

- Congenital anomaly: a structural or functional anomaly of organs, systems or parts of the body that occurs during intrauterine life and is caused by genetic or environmental factors (e.g. exposure to toxic substances, micronutrient deficiencies or maternal diseases), or both.
- Developmental toxicant: a chemical that causes adverse effects on the developing organism, including death, structural abnormality, altered growth and functional deficiency, or any combination thereof.
- Developmental no-effect level (dNOEL): the highest dose at which no embryotoxicity is observed.
- Developmental low-effect level (dLOEL): the lowest dose at which embryotoxicity is observed.
- Embryo: the term given to the product of conception from implantation through the first 8 weeks after conception (equivalent to 10 weeks of gestation computed from the day of the last menstrual period) in humans.
- Embryotoxicity: any adverse effect on the developing embryo, including death (embryo lethality) and developmental effects.
- External congenital anomaly: a type of anomaly that can be identified by inspection during physical examination.
- Fetal death: fetal death refers to death prior to the complete expulsion or extraction of a product of conception from its mother, irrespective of the duration of pregnancy; the death is indicated by the fact that, after such separation, the fetus does not breathe or show any other evidence of life, such as beating of the heart, pulsation of the umbilical cord, or definite movement of voluntary muscles.
- Fetus: an unborn baby from the 8th week after conception until birth.
- Gestational age: the time elapsed, measured in weeks, since the beginning of the woman's last normal menstrual cycle (i.e. ~2 weeks prior to conception).
- Gestation day: day of gestation considering the day of conception to be gestation day 0.
- Maternal toxicity: reduced food consumption and body weight gain, abortions, death and any other adverse effect directly on the mother.
- Minor congenital anomaly: a structural change that poses no significant health problem and tends to have limited social or cosmetic consequences for the affected individual.
- Miscarriage (synonym to spontaneous abortion): a spontaneous loss for a clinical pregnancy before 28 completed weeks of gestational age.
- Pregnancy outcome: the result of conception and ensuing pregnancy, including live birth, stillbirth, miscarriage and induced abortion.

- Selective developmental toxicant: a chemical that has a developmentally toxic effect at doses lower than its maternally toxic effect.

- Stillbirth: WHO defines stillbirth as third-trimester fetal death (1000 grams or more; 28 weeks or more) for international comparison purposes. However, in broader terms, a stillbirth is a fetal death after the gestational age of viability. The definition of viability is based on gestational age and/or weight and is variable among countries.

- Teratogen: an agent capable of interrupting or altering the normal development of an embryo or fetus, often resulting in a congenital anomaly or embryonic or fetal death.

# Executive summary

Malaria in pregnancy is a significant health problem in malaria-endemic areas. It not only causes substantial childhood morbidity and mortality but also increases the risks of adverse events for pregnant women and their developing fetuses. Most of the burden in these areas is due to infection with *Plasmodium falciparum*. Since 2006, artemisinin-based combination therapy (ACT) has been recommended as first-line treatment for uncomplicated *P. falciparum* malaria in all populations, including pregnant women in their second and third trimesters. Until late 2022, the World Health Organization (WHO) recommended as first-line treatment a combination of quinine (QNN) and clindamycin for treatment in the first trimester.

The artemisinins were initially not recommended for the treatment of uncomplicated malaria in the first trimester because of embryotoxicity in animal studies, as well as a lack of data in humans. The exceptions were cases of severe malaria or as second-line treatment[1] if QNN and clindamycin were not available or failed. However, a systematic review and meta-analysis on artemisinin-based treatment of pregnant women in the first trimester in South-East Asia and sub-Saharan Africa provided some reassurance that the animal findings do not directly translate to humans. The meta-analysis showed no difference in the risks of miscarriage, stillbirth or major congenital anomalies between pregnancies with first-trimester exposures to artemisinins and pregnancies with non-artemisinin-based treatment, including treatment with QNN. Furthermore, first-trimester treatment with artemether–lumefantrine (AL) was associated with significantly fewer (42% lower) adverse pregnancy outcomes than first-trimester oral QNN treatment.

Based on the review of the evidence in 2022, the *WHO guidelines for malaria* published in November 2022 recommend AL, the ACT with the most human safety data available, as the preferred treatment for uncomplicated *P. falciparum* malaria in the first trimester of pregnancy, replacing oral QNN regimens. Although limited data are available on specific ACTs other than AL, other ACTs – artesunate–amodiaquine (AS-AQ), artesunate–mefloquine (AS-MQ) and dihydroartemisinin–piperaquine (DHA-PPQ) – may be considered for use where AL is not recommended or is unavailable. Antifolates are contraindicated in the first trimester of pregnancy because of concerns about the increased risk of neural tube defects and therefore ACTs containing sulfadoxine–pyrimethamine (SP) are contraindicated in the first trimester. At the time of the review by WHO, there was no documented record of the use of artesunate–pyronaridine (AS-PYR) during the first trimester of pregnancy and therefore AS-PYR is not currently recommended. The recommendation of AL as the preferred treatment takes into account the demonstrated poorer outcomes of QNN treatment, along with the challenges of adherence to a 7-day course of treatment.

This document presents all relevant evidence on the effects and safety in early pregnancy of artemisinins and partner medicines used in ACTs from both studies in experimental animals and observational studies in humans.

### Reproductive toxicology of artemisinins in experimental animals

Artemisinin derivatives are embryotoxic in all species studied (rats, rabbits and monkeys) at low doses, similar to the human equivalent dose (HED), in the absence of maternal toxicity. Studies in monkeys showed that artesunate (AS) is embryolethal when dosed for at least 12 days starting on gestation day (GD) 20 but not when dosed for 3 days from GD 29 or 7 days from GD 27, indicating that developmental toxicity of AS is dependent on the duration of dosing and/or the period after gestation at which administration begins.

1 An ACT or oral artesunate + clindamycin was considered an alternative if quinine + clindamycin failed or was not available.

Preclinical studies showed that the mechanism of embryotoxicity was damage to immature red blood cells (primitive erythroblasts), which caused severe anaemia in the embryos, leading to either death, or skeletal malformations (shortened or bent long bones and scapulae, misshapen ribs and cleft sternebrae) and cardiovascular malformations (ventricular septal and vessel defects).

The studies predicted that the main window for any effects on the embryo would be early in pregnancy, corresponding to approximately 4–10 weeks post-conception in humans, which is the period when nucleated, metabolically active primitive erythroblasts predominate in the blood.

**Reproductive toxicology of clindamycin, QNN and non-artemisinin ACT partner medicines in experimental animals**

Clindamycin was shown to have no embryotoxicity in pregnant rats or mice. QNN and four of the antimalarial medicines (lumefantrine, mefloquine [MQ], pyrimethamine and sulfadoxine) that are combined with artemisinins in recommended ACTs caused embryo deaths and/or malformations in at least one animal species. QNN and two of the partner medicines (MQ and pyrimethamine) were embryotoxic at HEDs close to or below therapeutic doses. QNN specifically affected the development of the brain and inner ear in rabbits.

In terms of ACT partner medicines, MQ was embryotoxic at doses that were not toxic to the dam (i.e. a selective embryo toxicant) in rabbits and mice, in which the primary effects were embryo deaths and, in mice only, cleft palate. Lumefantrine was embryotoxic in rats and rabbits, but only at relatively high doses and when administered in combination with artemether. With lumefantrine monotherapy, no maternal or developmental findings were observed in rats or rabbits. Amodiaquine (AQ) and piperaquine (PPQ), had only minor developmental effects (variations) in rats. Pyronaridine (PYR) caused embryo deaths in rabbits, but only at a dose that was excessively toxic to the dams. Pyrimethamine induced malformations in rats, minipigs and mice. Sulfadoxine induced cleft palate in rats. The sulfadoxine and pyrimethamine combination led to embryo death in rats and rabbits and, in rats only, cleft palate.

**Safety of artemisinin compounds in the first trimester of human pregnancy**

A systematic review and meta-analysis were conducted for a recent WHO review of the safety of artemisinin when given in the first trimester of pregnancy. The risk of adverse pregnancy outcomes in prospective observational studies of pregnant women in Africa and Asia was analysed by comparing women exposed to artemisinin-based treatment (ABT) during the first trimester with those who received non-artemisinin-based treatment (non-ABT) or no antimalarial treatment. A total of 34 178 pregnancies in seven studies (12 cohorts across nine countries: eight in sub-Saharan Africa and one in Asia) were analysed using individual patient data; antimalarial treatment was confirmed by additional data sources such as clinic cards and outpatient registers. The primary end-point was a composite adverse pregnancy outcome, comprising miscarriage, stillbirth and major congenital anomaly.

No difference was observed in the risk of adverse pregnancy outcomes associated with the use of ABT at any time during the first trimester ($n$ = 736) compared with non-ABT ($n$ = 1074; adjusted hazard ratio [aHR] 0.71; 95% confidence interval [CI]: 0.49–1.03). Similar results were seen for the individual components: miscarriage (ABT: 27/669 [4.0%]; non-ABT: 76/1070 [7.1%]; aHR 0.74; 95% CI: 0.47–1.17), stillbirth (ABT: 13/646 [2.0%]; non-ABT: 12/743 [1.6%]; aHR 0.71; 95% CI: 0.32–1.57) or major congenital anomalies (ABT: 2/736 [0.3%]; non-ABT: 8/1074 [0.7%]; aHR 0.60; 95% CI: 0.13–2.87). The risk of adverse pregnancy outcomes was lower with AL than with oral QNN in the first trimester (25/524 [4.8%] vs 84/915 [9.2%]; aHR 0.58; 95% CI: 0.36–0.92). AL was the only ACT with sufficient data for a subgroup analysis.

There was also no difference in the risk of adverse pregnancy outcomes in an analysis restricted to exposure during the putative embryo-sensitive period for artemisinins (6–12 weeks of gestation) between ABT and non-ABT (ABT: 37/584 [6.3%]; non-ABT: 60/823 [7.3%]; aHR 0.95; 95% CI: 0.63–1.45).

In comparison with women not treated with any antimalarial (considered not to have malaria) in the first trimester, those treated with non-ABT had a higher risk of adverse pregnancy outcomes (aHR 1.30; 95% CI: 1.06–1.60), while those treated with ABT did not (aHR 0.92; 95% CI: 0.67–1.26). These findings suggest that prompt treatment with effective ABT antimalarials can counteract some of the adverse effects of malaria infection in early pregnancy and did not trigger adverse pregnancy outcomes compared with non-ABT.

**Differences in embryotoxicity between animal and human data: possible explanations**

Differences between animal studies and human data regarding the safety of artemisinin derivatives during early pregnancy may be explained by several potential factors. First, the timing of exposures in relation to the putative embryo-sensitive period when primitive erythroblasts are in circulation, as well as interspecies variations in embryo–fetal erythropoiesis development, must be considered. Primitive erythroblasts, the target of artemisinin embryotoxicity, are formed over a longer period in humans (~6 weeks) compared to animals (e.g. 4 days in rats) where these are produced over a very limited time frame and a single exposure can lead to a significant proportion of cell deaths without time for replacement. Consequently, the typical malaria treatment duration of 3–7 days with ACTs or artemisinin derivatives monotherapy (e.g. for severe malaria) may not result in irreversible harm in humans as has been observed in rodents. Even if the dose level administered would produce a transient reduction in the primitive erythroblast population in the human embryo, the population may be replenished by newly generated cells, resulting in marginal toxic effects with no discernible clinical implications. This is supported by the findings from studies in monkeys, which showed embryotoxicity following 12 days of AS treatment but none when treatment was restricted to 3 or 7 days. Secondly, the molecular target of artemisinins is unknown and could differ between animals and humans. Lastly, it has been hypothesized that malaria infection could reduce drug distribution to the fetus and mitigate the embryotoxic effects of certain antimalarials (including artemisinin and QNN). However, it remains uncertain whether the limited parasite biomass and quantity of drug sequestered within parasitized red blood cells can account for the observed variations in embryotoxicity between animal and human studies. Further investigations are needed to evaluate possible artemisinin-induced embryotoxicity in humans and whether malaria protects against it. The benefit–risk balance for the use of ACTs for chemoprevention in the first trimester has not been evaluated, and the current WHO recommendations relate specifically to case management of malaria

**Safety of non-artemisinin ACT partner medicines in the first trimester of human pregnancy**

Little information is available on the risks associated with non-artemisinin ACT partner medicines used in early human pregnancy, except for MQ. More than 1000 first-trimester exposures to MQ have been documented, and recent reviews have concluded that MQ can be used safely throughout pregnancy. AQ is considered safe in pregnancy, although there is limited documented use in the first trimester ($n$ = 78, including 67 as a combination with AS). All documented exposure to lumefantrine has been in combination with artemether ($n$ = 575). The meta-analysis described above, with more than 500 AL exposures in the first trimester, showed no evidence of teratogenicity or embryotoxicity based on the risk of miscarriage, stillbirth or major congenital anomalies compared with QNN. There are limited data on the use of PPQ (252 exposures to DHA-PPQ in the first trimester). However, a recent unpublished study from Indonesia showed no increase in the risk of pregnancy

loss (miscarriage or stillbirth) or major congenital anomalies among 159 pregnant women treated with DHA-PPQ in the first trimester compared with those treated with QNN. Pyrimethamine and sulfadoxine are antifolate agents, and other members of this class are probable human teratogens. There is limited evidence of the use of SP ($n$ = 300) and very limited information on the effects of treatment with PYR in the first trimester of pregnancy ($n$ = 6).

**Conclusions**

The results of the meta-analysis of human first-trimester exposures to artemisinin are reassuring and suggest that the findings from animal studies do not directly translate to humans. They indicate that in comparison with antimalarials considered safe in the first trimester, including QNN, treatment with an artemisinin-based regimen in the first trimester of pregnancy is not associated with increased risk of miscarriage, stillbirth or major congenital anomalies. Furthermore, treatment in the first trimester with AL was associated with a 42% lower risk of adverse pregnancy outcomes than treatment with oral QNN. This evidence has been used to update the recommendations in the *WHO guidelines for malaria*, with AL as the preferred option for the treatment of uncomplicated malaria in women in the first trimester of pregnancy. Ensuring the safety of antimalarial treatments during pregnancy is critical, and it is imperative to continue generating robust evidence through active pharmacovigilance and clinical trials, particularly for antimalarial drugs administered during the first trimester.

# 1. Introduction

The World Health Organization (WHO) first recommended the use of artemisinin-based combination therapies (ACTs) in places where they are more efficacious than available monotherapies in November 2000 *(4)*. In 2001, a WHO report *(5)* concluded: "Pre-clinical studies have shown that artemisinin and its derivatives do not exhibit mutagenic or teratogenic activity. However, the drugs have caused fetal resorption in rodents at relatively low doses of >10 mg/kg, when given after the sixth day of gestation. Reports on the use of these drugs in humans during pregnancy are limited." Thus, "because of the effects in rodents and the very limited data in humans" *(5)*, the artemisinin derivatives were initially not recommended for use in the first trimester of pregnancy". In 2002 and 2006, as research progressed and more countries adopted ACTs as first-line treatment for malaria, the United Nations Children's Fund (UNICEF)–United Nations Development Programme (UNDP)–World Bank–WHO Special Programme for Research and Training on Tropical Diseases (TDR) convened two informal consultations, involving preclinical and clinical experts, on the safety of artemisinins in pregnancy *(6, 1)*. Because of the lack of data on human exposure at that time, both consultations concluded that artemisinin compounds could not be recommended for treatment of malaria in the first trimester of pregnancy.

Additional studies have been conducted during the past 16 years, including several studies of pregnancy registries in South-East Asia and sub-Saharan Africa; furthermore, the wider use of ACTs as first-line treatment has significantly increased exposure to these medicines. The new evidence was reviewed by WHO in July 2015 and April 2022. A systematic review and meta-analysis served as the basis for updating the WHO recommendation on the use of artemisinins in the first trimester of pregnancy.

For this document, the authors reviewed all the available evidence to assess the safety of artemisinins and ACT partner medicines in early pregnancy. They discuss the methods and challenges in assessing drug safety in pregnant women and the basis for the WHO recommendations presented in the guidelines for the treatment of malaria. They also evaluated published studies on the reproductive and embryo toxicity of artemisinin and non-artemisinin ACT partner medicines in experimental animals. The authors present the results of a comparative analysis of studies of the effects of exposure to artemisinin and to quinine in the first trimester of human pregnancy. The conclusions are based on all the data reviewed.

All authors completed a "Declarations of Interests for WHO experts" form. WHO processes were used to assess declared interests and none of the authors were deemed to have conflicts of interests in relation to the completion of this review.

This document completes the series of documents published by TDR in 2003 *(6)* and 2007 *(4)*, and presents the evidence base for current WHO recommendations on the case management of uncomplicated malaria in the first trimester of pregnancy: "Pregnant women with uncomplicated *P. falciparum* malaria should be treated with artemether–lumefantrine during the first trimester" *(7)*.

# 2. Background

## 2.1 Malaria in pregnancy

Pregnant women are at greater risk than non-pregnant women and the general population of becoming infected with malaria parasites and developing symptomatic and complicated malaria.

Malaria in pregnancy is associated with increased maternal, fetal and neonatal morbidity and mortality. The adverse consequences for the mother and her developing fetus include severe maternal anaemia, intrauterine growth retardation, intrauterine death, stillbirth, premature delivery and low birth weight, as well as increased risks of miscarriage, severe malaria and maternal mortality in low-transmission areas *(8–12)*.

In high-transmission settings, where levels of acquired immunity tend to be high, *Plasmodium falciparum* infection is usually asymptomatic in adults – both men and women. For unclear reasons, pregnancy reduces a woman's immunity to malaria; pregnant women consequently become more susceptible to infection, with increased risks of illness and adverse effects for the newborn, particularly in primigravidae and secundigravidae. The gravidity effect is less pronounced in low-transmission areas. Sequestration of parasite-infected erythrocytes in the placenta and inflammatory cells contributes to maternal anaemia, which, with maternal parasitaemia, can contribute to low birth weight, an important risk factor for infant mortality.

The World Health Organization (WHO) recommends a three-pronged approach to controlling malaria in pregnancy *(13)*: use of insecticide-treated bednets; prompt, effective case management, comprising timely diagnosis and adequate treatment; and, in all areas of moderate to high malaria transmission in Africa, provision of intermittent preventive treatment with sulfadoxine–pyrimethamine (SP). Unless they sleep under insecticide-treated nets, most women are not protected from malaria in early pregnancy. Intermittent preventive treatment with SP is contraindicated in the first trimester *(14)*. Although pregnant women are commonly given nets at antenatal care clinics in sub-Saharan Africa, they usually present for their first visit late in their second trimester *(15)*.

Pregnant women with malaria in their first trimester who are not adequately treated are at high risk for placental malaria infection *(16)*. However, even infections with abundant sequestered placental parasites are often not detected because microscopy examination of peripheral blood smears often fail to detect infection, resulting in an incorrect diagnosis and therefore no treatment, which contributes to adverse pregnancy outcomes. A growing body of evidence highlights the burden of malaria infection in early pregnancy and its associated poor maternal and infant outcomes, including hypertensive disorders during pregnancy, maternal anaemia, pregnancy loss, preterm birth, intrauterine growth retardation and low birth weight *(17–27)*. *P. falciparum* infections early in pregnancy impair placental vasculogenesis and angiogenesis, and affect the ability of the placenta to support fetal growth *(22, 27–30)*. Furthermore, this is the period of pregnancy when women are at the highest risk of infection *(31–34)*. A modelling study estimated that 65% of women affected by malaria in pregnancy acquire the infection during the first trimester *(35)*.

A balance should be found between the risks associated with malaria and the risks associated with antimalarial treatment in pregnancy, involving effective case management.

The antimalarials recommended for pregnant patients must be safe for both the mother and the fetus. However, most medicines are not tested in pregnant women before they become available to patients, primarily because of concern that they will harm the mother and/or the embryo or fetus, particularly if given during the first trimester of pregnancy when organogenesis takes place. Because of the lack of clinical studies in pregnant women, most medicines are not recommended during pregnancy and are consequently generally not prescribed, or prescribed with limited information on the risk to the mother, her pregnancy and the fetus. Due to these significant evidence gaps, potentially useful and effective medicines are often restricted in use in pregnancy, particularly in the first trimester when pregnant women are commonly treated with older and often less effective drugs.

## 2.2 Methods and challenges in assessing the safety of antimalarial medicines in pregnancy

Pregnant women are routinely excluded from clinical trials so post-marketing drug surveillance is critical for detecting fetal effects. The safety of medicines in pregnancy is better assessed by active rather than passive surveillance, as recommended by both the European Medicines Agency *(36)* and the United States Food and Drug Administration *(37, 38)*.

Pregnancy exposure registries can be used for prospective identification and follow-up of exposed women until the end of pregnancy. Use of registries reduces selection and recall bias, and provides risk estimates that can be compared with those of appropriate control groups or background population rates *(39)*.

In high-income countries, information on the safety of medicines used in pregnancy can be derived from medical records and automated databases, including medical and pharmacy insurance claims *(40, 41)*. This is generally not feasible in low-income countries with limited pharmacovigilance or automated data sources. As a result, data on medicines safety from industrialized countries are often used, but these usually include minimal information on medicines used to treat tropical diseases *(42)*.

The methodological challenges to studying the safety of medicines in pregnancy include those common to all pharmacovigilance studies, such as the requirement for large samples to minimize the possibility that any observed association between a medicine and a rare outcome is due to chance. Studies should also consider the contribution to risk from underlying maternal infection or illness, the general lack of data on background rates of adverse outcomes and the potential bias introduced by voluntary reporting *(43)*. Monitoring medicine safety in pregnancy involves systematic assessment and recording of maternal and newborn outcomes, ideally with follow-up of infants to identify anomalies and learning disabilities that are not detectable at birth. Systems should be established to record all pregnancy outcomes of deliveries outside health-care facilities, particularly to detect early pregnancy loss; deliveries often take place outside health-care facilities in rural settings in low- and middle-income countries. This requires early detection and enrolment of pregnancies. Furthermore, assessing congenital anomalies in newborns requires careful examination by trained staff using standard tools and definitions *(44, 45)*.

Ascertainment of drug exposure requires reliable information on the medicines used and the gestational age at the time of exposure. The impact on the fetus of medicines used in pregnancy depends on the stage of pregnancy at the time of exposure, as different tissues and organs develop at different times *(46)*. Retrospective determination of the precise time of exposure is difficult, especially when the treatment course is short (e.g. 3 days for artemisinin-based combination therapy [ACT]), and a multi-pronged approach is required to obtain the details of exposure.

Methodological considerations in the assessment of the safety of ACTs in pregnancy have been described in detail elsewhere *(45, 47, 48)*. These publications suggested methods and protocols for assessing the safety of medicines in pregnancy in malaria-endemic and resource-limited countries. Because of the methodological and ethical challenges of including first trimester pregnancy in treatment trials without enough data to determine risks to the mother, her pregnancy and the embryo or fetus, limited evidence has been generated on the safety and efficacy of ACTs in early pregnancy since their introduction more than 20 years ago.

## 2.3 WHO guidelines on management of malaria in pregnancy

Until recently, WHO recommended only the following antimalarial agents for use in the first trimester of pregnancy: quinine (QNN)-clindamycin, chloroquine and mefloquine (MQ). While the combination of QNN with clindamycin is suggested to augment effectiveness, QNN monotherapy is frequently prescribed, primarily because clindamycin is often inaccessible in resource-limited malaria-endemic regions due to its relatively high cost. A review of 35 national malaria treatment guidelines suggests that two thirds recommend QNN monotherapy in the first trimester *(49)*. QNN has several disadvantages, including its long and complex treatment course (8-hourly for 7 days) and poor tolerability, with the associated high risk of non-adherence which would result in incomplete treatment. Furthermore, chloroquine is no longer effective against falciparum malaria in many areas and MQ monotherapy is not recommended due to the threat of drug resistance. ACTs are among the most effective, rapidly acting antimalarials available, saving the lives of children and adults, including women in the second and third trimesters of pregnancy; they are currently the first-line treatment for malaria in most endemic countries *(50)*. ACTs are combinations of a fast-acting artemisinin derivative with a slower-acting, structurally unrelated antimalarial. For uncomplicated *P. falciparum* malaria infections in non-pregnant women, WHO recommends the following ACTs given daily for 3 days: artemether–lumefantrine (AL) 80 + 480 mg twice daily dose for adults; artesunate–amodiaquine (AS-AQ) 200 + 540 mg daily dose; artesunate–mefloquine (AS-MQ) 200 + 440 mg daily dose; AS + SP 200 mg daily dose + 1500 + 75 mg dose on first day of treatment; dihydroartemisinin–piperaquine (DHA-PPQ) 160 + 1280 mg daily dose up to 80 kg body weight or artesunate–pyronaridine (AS-PYR) 240 + 720 mg daily *(5, 51)*. The existing ACTs administered over 3 days include a target daily dose and range of 4 and 2–10 mg/kg for artesunate and dihydroartemisinin, and a target daily dose range of 1.6–8 mg/kg for artemether.

From 2006 to November 2022, the WHO recommendation was to "treat pregnant women with uncomplicated *P. falciparum* malaria during the first trimester with 7 days of QNN + clindamycin (strong recommendation)" *(52)*. Until now, use of ACTs in the first trimester has been restricted to situations in which it is the only treatment immediately available, treatment with QNN + clindamycin fails, or poor adherence to a 7-day treatment course is predicted. The restriction on treatment with ACTs for pregnant women in the first trimester was based on evidence from studies in experimental animals that artemisinin derivatives are toxic to embryos and on the lack of adequate data on safety for women in the first trimester of pregnancy. However, inadvertent exposure to ACT in the first trimester for confirmed or presumed malaria is very likely if the pregnancy is not known or disclosed, or when health-care providers are unaware of the guidelines *(53, 54)*. Furthermore, many surveys in Africa have shown that there is little diagnostic testing for malaria in the private sector, where a large proportion of the population, especially adults, seek treatment *(55)*.

In 2002 and 2006, WHO convened informal consultations among preclinical and clinical experts to review the most recent findings and assess the safety of giving artemisinin

derivatives in pregnancy. The conclusion of the 2002 meeting *(3)* was that "Presently, artemisinin compounds cannot be recommended for treatment of malaria in the first trimester". The conclusion of the 2006 meeting *(4)* was that "There is insufficient evidence at present to warrant a change in current WHO policy recommendations on the use of artemisinin-based products for the treatment of malaria in pregnancy". The WHO guidelines developed in 2006 recommended that, in uncomplicated malaria, ACT should be used in the second and third trimester, but should be used in the first trimester only if it is the only effective treatment available.

A summary of studies of the embryotoxicity of artemisinins in experimental animals and humans conducted since 2006 was prepared for presentation at a WHO meeting in July 2015 *(56)*. The meta-analysis summarizing evidence on human exposures was updated in 2022 *(57)* and presented to the WHO Guideline Development Group. Based on the review of the evidence by the Guideline Development Group, the WHO *Guidelines for the treatment of malaria* were revised to recommend AL as the preferred treatment for uncomplicated *P. falciparum* malaria in the first trimester of pregnancy *(5)*. The text of the new treatment guidelines is shown in the text box below.

**New WHO recommendation on malaria case management in pregnancy *(5)***

**Pregnant women with uncomplicated *P. falciparum* malaria should be treated with artemether–lumefantrine during the first trimester.**

- Limited exposures to other ACTs (artesunate–amodiaquine, artesunate–mefloquine and dihydroartemisinin–piperaquine) suggest that the current evidence is insufficient to make a recommendation for routine use of these other ACTs in the first trimester of pregnancy. However, consistent with the previous WHO recommendation that provided for limited use of ACTs if the first-line recommended medicine was not available, these other ACTs may be considered for use where artemether–lumefantrine is not a recommended ACT for uncomplicated malaria or is not available, given the demonstrated poorer outcomes of quinine treatment, along with the challenges of adherence to a seven-day course of treatment.
- Antifolates are contraindicated in the first trimester of pregnancy. Therefore, ACTs containing sulfadoxine–pyrimethamine are contraindicated during the first trimester of pregnancy.
- There is currently no documented record of the use of artesunate–pyronaridine during the first trimester of pregnancy.
- Continued pharmacovigilance and clinical research, including prospective controlled trials on the efficacy and safety of antimalarial medicines for the treatment of malaria in pregnancy, should be supported and funded.

Strong recommendation for, low certainty evidence (2022)

# 3. Assessment of embryotoxicity and reproductive risk of artemisinins in pregnant animals

**Studies of early embryo–fetal development in rats and rabbits with dosing throughout organogenesis showed that AS could induce embryo malformations and fetal death at low non-maternally toxic oral doses. Malformations were caused in rats at a dose of 7 mg/kg per day, representing a human equivalent dose (HED) of 1.0 mg/kg per day, and in rabbits at 5 mg/kg per day, representing a HED of 1.6 mg/kg per day *(58)*, both HEDs being below the therapeutic dose. In rats, the sensitive period was GD 10 to 14, with GD 10 being the most sensitive day for the induction of malformations and GD 11 being the most sensitive day for the induction of embryo death *(59)*.**

**These findings led to studies in pregnant monkeys with varying durations of dosing *(60)* and studies in rats with single doses *(61)*. The summary of studies of embryotoxicity in experimental animals exposed to artemisinins in pregnancy that was prepared for the WHO evidence review group in 2015 was updated in July 2019 using the same search strategy and published in 2020 *(62)*.**

**The method for the literature review is described in the publication of González *et al*,. 2020 *(62)*. Particular attention was paid to original articles, and additional references were obtained from the references in the articles identified in the search. An updated search in June 2023 did not yield new studies investigating artemisinin effects on pregnant animals.**

**The criteria for inclusion in the review were publication between 2007 and 2019, and reporting on the effects of artemisinin derivatives on embryonic development in pregnant animals. Studies on the effects of *Artemisia annua* plants were not included. Of 194 articles found, 20 were selected, of which 18 met the inclusion criteria and were included in the review. Seventeen studies were conducted in rats, two of which also included rabbits, and one study was in monkeys.**

## 3.1 Duration of dosing and therapeutic dosing regimen

The studies of artemisinins in pregnant animals included both studies conducted to support regulatory submission (animals were treated throughout organogenesis) and a series of investigative studies with short treatment periods (as short as for a single day). For example, the regulatory study of AS in rats involved treatment on GD 6–17 whereas the regulatory study of AS in rabbits involved treatment on GD 7–19 *(58)*. In humans, the duration of organogenesis is approximately 46 days *(63)* and the duration of oral treatment with artemisinin-containing combinations is generally 3–7 days. Thus, the studies which involved treatment on single days of gestation *(59, 61)* better reflect the clinical situation.

## 3.2 Exposure ratios and HEDs

Animal-to-human exposure ratios are used to assess the risk of a compound during pregnancy. When data on pharmacokinetics are available, the ratio can be determined of an exposure metric in the animal species – such as the area under the curve (AUC) of concentration versus time or the maximum concentration (Cmax) achieved at the highest

dNOEL – to the metric at the therapeutic dose in humans. Even when pharmacokinetics data are not available, the doses used in animals (at the dNOEL and/or dLOEL) and the human therapeutic doses can be compared by allometric scaling based on dose per body surface area (i.e. mg/m2), which allows adjustment for species differences in physiological parameters *(64–68)*. First, the HED is calculated by dividing the dose used in animals 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 *(65)*). The animal HED at the dNOEL or dLOEL is then divided by the human therapeutic dose to obtain the HED ratio. Referring specifically to AUC and Cmax ratios, Guideline S5 (R3) 2020 from the International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use *(69)* states: "In general, there is increased concern when the NOAEL [no adverse effect level] occurs at exposures less than 10-fold the human exposure at the MRHD [maximum recommended human dose]; above this threshold, concern is reduced. Effects that are limited to occurrence at more than 25-fold the human exposure at the MRHD are usually of minor concern for the clinical use of the pharmaceutical."

## 3.3 Embryotoxicity in rats and rabbits

Artemisinins caused teratogenicity and embryo death in rats even when administered as single doses in the absence of maternal toxicity *(61)*.

When administered throughout organogenesis, AS caused embryo death at the lowest dose tested in rats (6 mg/kg per day; HED = 0.97 mg/kg) and at 12 mg/kg per day in rabbits (HED = 3.9 mg/kg), both in the absence of maternal toxicity *(58)*. In a study in which rats were treated at doses of AS of 2, 4 and 8 mg/kg per day *(70)*, increased incidences of embryo death, including totally resorbed litters, and of visceral and skeletal variations were seen at the two higher doses. The dose of 2 mg/kg per day was the dNOEL.

In a study in which single doses of 17 mg/kg AS (HED = 2.7 mg/kg) were administered to rats on different days of gestation, the period GD 10–14 was found to be sensitive with at least a 29% incidence of embryo deaths on each day compared with 4% in the control *(59)*. The most sensitive day for the induction of embryo death was GD 11, when a single dose of 10 mg/kg AS (HED = 1.6 mg/kg) caused 15% embryo deaths and a single dose of 17 mg/kg (HED = 2.7 mg/kg) caused 100% embryo deaths (both in the absence of maternal toxicity) compared with 3–4% in the control. The most sensitive day for the induction of malformations was GD 10, when a single dose of 17 mg/kg AS (HED = 2.7 mg/kg) caused a 17.5% incidence of ventricular septal defects (in the absence of maternal toxicity) compared with 0% in the control and, at the dNOEL of 10 mg/kg, the AUC ratio was <1 *(71)*.

The pattern of embryotoxicity observed originally with AS is also seen with the other most used artemisinins: DHA and artemether *(61)*. When artemether was administered to rats on GD 7–14, it caused 32% embryo deaths at a dose of 3.5 mg/kg per day (HED = 0.6 mg/kg) and 100% embryo deaths at a dose of 7 mg/kg per day (HED = 1.1 mg/kg) in the absence of maternal toxicity – visceral and skeletal examinations were not performed *(72)*. When the drug artemisinin was administered to rats on GD 7–13, it caused 100% embryo loss in the absence of maternal toxicity at 35 and 75 mg/kg per day (HEDs = 5.6 and 12.1, respectively); it had a slightly lower effect (87% embryo loss) when administered at 35 mg/kg per day on GD 14–20 – fetal examinations were not performed *(73)*. In a single dose study, 15 mg/kg AS (HED = 2.4 mg/kg), 11.1 mg/kg DHA (HED = 1.8 mg/kg) and 19.4 mg/kg artemether (HED = 3.1 mg/kg) were administered orally to rats on GD 10 in the absence of maternal toxicity. These treatments caused 70%, 55%, and 69% embryo deaths, respectively, (compared to 6% in the control) and 31%, 37% and 58% incidences of ventricular septal defects, respectively, (compared to 0% in the control) and similar incidences of knobby rib and other anomalies *(61)*. Thus, all these artemisinins caused the same pattern of embryo death and fetal cardiovascular and skeletal abnormalities

in the absence of maternal toxicity *(61, 74)*. These studies indicated that DHA may be the proximate developmental toxicant in rats.

In another study, AS was associated with an adverse effect on bone growth. After a dose of 4 mg/kg per day administered to pregnant rats on GD 9–11, there was a 30% decrease in fetal weight and more marked decreases in the size of the skeleton, including the femur, tibia and humerus *(75)*. However, no embryonic death was observed when dosing was confined to GD 9–11. After intravenous or intramuscular injection of AS at 1.2 mg/kg per day for 5 days, the order of timing for increasing incidence of embryo death was GD 11–15 > GD 16–20 > GD 6–10 *(76)*.

Studies indicate that AS and DHA might be associated with a higher risk of embryotoxicity compared with artemether and arteether. Additionally, the mode of administration may influence this risk, with injectable AS showing a higher risk of embryotoxicity than oral AS *(77)*.

## 3.4. Embryotoxicity in monkeys

AS was embryolethal in cynomolgus monkeys at 12 mg/kg per day (HED = 3.9 mg/kg) and 30 mg/kg per day (HED = 9.7 mg/kg) when given for at least 12 days from GD 20 *(60)*. In the 30 mg/kg per day group, three live embryos were collected on GD 26, 32 and 36 and examined histologically. All three embryos had marked reductions in nucleated embryonic erythroblasts, which were likely primitive erythroblasts. However, when the potentially embryotoxic dose of 12 mg/kg per day was given for only 3 or 7 days (on GD 29–31 and GD 27–33, respectively), no embryotoxicity was seen. There were no maternal effects in any of these groups except for a mild decrease in food consumption during GD 23–27 in the group treated with 30 mg/kg per day for at least 12 days. The authors concluded that the embryotoxicity of AS depends on the duration of dosing and/or the period of pregnancy.

## 3.5. Cellular target for artemisinin-induced embryotoxicity

AS-induced embryotoxicity in rats appears to result from sustained depletion of circulating primitive erythroblasts, leading to anaemia, hypoxia and eventual cell death *(4, 78, 79)*. Similar observations were made in the study of monkeys described above *(60)*, in which three live embryos from mothers given 30 mg/kg per day starting on GD 20 were removed on GD 26, 32 and 36. Histological analysis showed a relative lack of blood cells in the vasculature and thinning of heart walls. In a study to localize the damage caused by AS and its derivatives in pregnant rats and fetuses, whole-body autoradiography showed that AS concentrated in tissues involved in haemoglobin synthesis and/or destruction in both the dam and the fetus *(80)*. This may account for the maternal reticulocytopenia and embryotoxicity observed with artemisinins. Monkey embryos also showed histological findings similar to those in rat embryos exposed to AS and DHA, in which the initial response was depletion of embryonic erythroblasts *(79)*.

Artelinic acid, a semi-synthetic artemisinin derivative not converted extensively to DHA, was found to have similar embryotoxicity to AS, killing circulating erythroid cells and stimulating maternal haematopoiesis *(81)*. This study also showed a strong correlation between embryo survival and reticulocyte count. A common feature of the target cells for lethal effects of artemisinins is mitochondria that produce haem. It has been hypothesized that both artelinic acid and artemisinins react with ferrous ions inside mitochondria to trigger cell apoptosis *(81, 82)*. It was subsequently shown that DHA arrests cell division and apoptosis of circulating embryonic erythroblasts in whole embryo culture *(83)*.

## 3.6. Relation of the sensitive period for embryo/developmental toxicity in animals to humans

In the study described above in which groups of rats were given single oral doses of AS on various days of gestation *(59)*, GD 10 was the most sensitive day for induction of fetal malformations, and GD 10–14 was a sensitive window for induction of embryo death within which GD 11 was the most sensitive day; this period is when nucleated primitive erythroblasts from the visceral yolk sac predominate in the embryonic circulation. Definitive erythroblasts originate from progenitor cells in the liver and start entering the circulation at about GD 13. In the human embryo, the onset of circulation begins around post-conception days 21–23.

In humans, primitive erythroblasts predominate in the circulation through post-conception week 8 but represent only about 5% of the circulating cells from post-conception week 11 *(84)*. The liver serves as the primary source of red cells from post-conception week 9, when it releases definitive erythroblasts *(85)*. If it is assumed that human embryos are sensitive to therapeutic doses of artemisinins, that the mechanism of embryotoxicity is the same in humans and rats, and that the embryo is sensitive until significant numbers of definitive erythroblasts have replaced primitive erythroblasts in circulation, the putative sensitive period in humans would be around post-conception weeks 4–10.

As previously mentioned, the embryotoxicity of AS depends on the duration of dosing and the period of pregnancy in which it is administered.

Table 1 shows the developmental events that occur during the estimated sensitive periods for artemisinin-induced embryotoxicity and the duration of organogenesis and pregnancy in rats and humans.

**Table 1. Putative sensitive periods for embryotoxicity in rats and humans**

| Developmental event[a] | Post-conception day | |
|---|---|---|
| | Rat | Human |
| **Conception** | 0 | 0 |
| **Organogenesis** | 5–15 | 15–56 (weeks 3–8)[b] |
| **Haematopoiesis in the yolk sac** | 9.5 | 18 |
| **Heartbeat** | 10 | 21–23 |
| **Start of definitive erythroblasts from liver in circulation** | 13 | 56[c] |
| **Birth** | 21–23 | 253–294 (weeks 37–42) |
| **Estimated period of sensitivity** | 10–14[d] | 22–70[e] (weeks 4–10) |

[a] Adapted from Clark *(74)*.
[b] From Donevan and Castella *(86)*.
[c] From Kelemen et al. *(84)* and Segel & Palis *(85)*.
[d] From Clark *(82)*.
[e] Period shown is the putative sensitive period estimated on the assumption that the events during the sensitive period in humans are correlated with the same events in rats.

## 3.7. Discussion

Extensive new information on the embryotoxicity of artemisinins has been published since 2007. The embryotoxic effects, which include embryolethality, malformations and decreased fetal weight, have been further characterized *(58–61, 72, 73, 79, 81–83, 87)*. Moreover, the embryotoxic effects seen primarily with AS have now been observed with other artemisinin derivatives, including artemether, DHA, and arteether, indicating a class effect in animal studies *(61, 73, 81, 88)*.

The sensitive period in rats has been established as GD 10–14, which corresponds developmentally to post-conception weeks 4–10 in human pregnancy (weeks 6–12 after the last menstrual period) *(59)*. The most sensitive days were GD 10 for the induction of fetal malformations and GD 11 for the induction of embryo deaths, which correspond to early in the putative sensitive period in human pregnancy. The most sensitive target for treatment on GD 10 in the rat was the closure of the ventricular septum, which occurs during the 5th week post-conception in humans *(63)*. AS has so far been reported to induce embryo death in three species (rat, rabbit and monkey) and, from the evidence described previously, there is likely to be a common cellular target. However, in monkeys embryotoxicity was observed only for extended duration of exposure (i.e. 12 days), which is longer than the duration of exposure required in humans for the treatment of malaria (i.e. 3 days). The developmental toxicity of AS depends on the duration of dosing and the period of pregnancy when treatment is given.

Artemisinins are reduced by the ferrous iron in haem to form carbon-centred free radicals that then bind covalently to haem, and this alkylation of haem is important for antimalarial activity *(89–94)*. Although synthetic trioxolane (therefore endoperoxide-containing) arterolane (OZ277) alkylates haem more efficiently than several artemisinins, including DHA *(89)*, it is less embryotoxic relative to its antimalarial activity than DHA *(95)*. Another synthetic endoperoxide, artefenomel (OZ439), is even less embryotoxic relative to therapeutic exposure than artemisinins (about 250 times less than DHA in whole embryo culture and about 100 times less than AS in rats in vivo) *(96)*. Studies with these trioxolanes therefore show that it is possible to largely separate the antimalarial activity of endoperoxide antimalarials from their embryotoxic activity. Overall, the evidence implies that while various artemisinin derivatives (AS, DHA, artemether and arteether) have shown embryotoxic effects in diverse animal species, the specific potential of each artemisinin derivative to induce embryotoxicity through relatively short exposure during pregnancy in animals or humans requires evaluation for each compound separately.

## 3.8. Conclusions

AS, artemether and DHA cause embryolethality and/or cardiovascular and skeletal abnormalities in the absence of maternal toxicity at low doses (corresponding to therapeutic doses in humans) in multiple animal species.

However, the embryotoxicity of artemisinins depends on the duration of dosing and the period of pregnancy when treatment is given. Extrapolating these animal reproductive toxicity findings to relatively short treatment courses (e.g. 3 days) for human exposure is complex, underscoring the necessity for human-based evidence to definitively validate or contest any inferences drawn from these animal inquiries. Subsequent sections will present the pertinent human data.

# 4 Assessment of embryotoxicity and reproductive risk of clindamycin, quinine and non-artemisinin ACT partner drugs in pregnant animals

**This section summarizes studies conducted in pregnant animals with QNN, clindamycin and non-artemisinin antimalarial partner medicines (AQ, lumefantrine, MQ, PPQ, PYR and SP). In general, the pregnant animal studies conducted involved administration during the period of organogenesis. A previous detailed review *(97)* included the outcome from the critical regulatory developmental toxicity studies. An updated literature search in September 2023 did not identify any new relevant articles.**

## 4.1 Summaries of studies in pregnant animals

### 4.1.1 Clindamycin

No embryotoxicity was observed in pregnant rats or mice with HED ratios at the dNOELs of 1.5 and 0.7, respectively. The product labels stated lack of teratogenicity at high doses by oral administration in studies in rats and mice that yielded high HED ratios (30 and 49, respectively) *(98, 99)*.

### 4.1.2 Quinine

QNN was shown to be embryotoxic in multiple animal species at HEDs close to or below human therapeutic doses. Brain and/or inner ear abnormalities were seen in rabbits *(100)*, chinchillas *(101)* and guinea pigs *(102)*, with HED ratios of approximately 0.03, 1.6 and 0.5, respectively. QNN-induced effects on embryo survival were observed in rabbits *(103)*, chinchillas *(101)*, mice *(104)* and dogs *(103)*, with HED ratios of approximately 1.6, 2.2, 1 and 0.4, respectively. In a definitive study in rats, no developmental effects were seen at a maternally toxic dose with a HED ratio of 0.8 and only minor developmental effects with a HED ratio of 1.7 *(105)*.

### 4.1.3 Amodiaquine

AQ did not cause embryo death or malformations in studies in rats and rabbits (R Davies, CIT , Study Number 28050 RSL, unpublished data, 2007; reviewed in 97), with HED ratios at dNOELs of 0.2 and 0.7, respectively. In rats, only minor developmental effects were seen at the dLOEL (HED ratio of 0.5).

### 4.1.4 Lumefantrine

Embryo–fetal development studies were conducted in rats and rabbits with lumefantrine given throughout organogenesis at a high dose of 1000 mg/kg per day *(106)*, which is generally considered the limit dose. In rats, litter size was decreased at this dose, but no developmental effects were seen at 300 mg/kg per day (dNOEL; HED ratio of 2.5). In rabbits, no developmental effects occurred, even at the limit dose of 1000 mg/kg per day (dNOEL; HED ratio of 17). Therefore, no adverse developmental effects were observed in animals at doses equivalent to the human therapeutic dose when lumefantrine is used as monotherapy. The exposure ratios for lumefantrine are more favourable than those for other antimalarials.

### 4.1.5 Mefloquine

At maternally toxic doses, MQ caused embryo deaths in rats, rabbits and mice, and malformations in rats (e.g. hydrocephaly) and mice (cleft palate) (*107*; T Short et al., Midwest Research Institute, unpublished data, 1976). In addition, MQ was a selective developmental toxin in rabbits and mice, with effects at doses that were not maternally toxic. When MQ is administered with AS for the treatment of malaria, the therapeutic dose is about 9 mg/kg per day, resulting in HED ratios at the dNOELs in rats of about 0.3 with treatment throughout organogenesis and 0.6 with treatment on GD 9–11. The HED ratio at the dNOEL was about 0.2 in both rabbits and mice.

### 4.1.6 Piperaquine

No unusual effects of PPQ on development were observed in embryo–fetal development studies in rats and rabbits *(108)*, in which the HED ratios at the dNOELs were 0.4 and 1.4, respectively. Reversible developmental effects (wavy rib and decreased fetal weight) occurred only in rats and only at maternally toxic doses.

### 4.1.7 Pyronaridine

PYR did not cause malformations in either rats or rabbits *(109, 110)*. Decreased fetal weight was observed in rats, but only at a high, maternally lethal dose. Effects on maternal food consumption and body weight gain were seen at the dNOEL (HED ratio of 2). Decreased embryo survival and decreased fetal weight were observed in rabbits, but only at a high, maternally lethal dose. The HED ratio at the dNOEL in rabbits was 1.2.

### 4.1.8 Sulfadoxine–pyrimethamine

Sulfadoxine and pyrimethamine are folate antagonists. When administered individually, pyrimethamine and sulfadoxine each caused embryo deaths and/or malformations in animals *(111, 112–119)*. Pyrimethamine induced malformations in rats, minipigs and mice. Sulfadoxine induced cleft palate in rats. The SP combination caused embryo deaths and cleft palate in rats, and embryo death in rabbits *(120, 121)*, with HED ratios of about 0.1 and 4, respectively.

## 4.2 Conclusions

QNN, which is among the non-artemisinin medicines recommended for use in uncomplicated malaria in the first trimester, caused malformations and embryo deaths in animals. Some of the non-artemisinin ACT partner medicines (MQ, pyrimethamine and sulfadoxine) caused embryo deaths and/or malformations in animals. Lumefantrine had higher HED ratios in animals (17 in rabbits and 2.5 in rats) than the other medicines discussed above. Lumefantrine resulted in decreased litter size only in rats and only at a relatively high dose (higher than a dose equivalent to the human therapeutic dose), and no malformations were observed. No adverse developmental effects were observed in animals at doses equivalent to the human therapeutic dose when lumefantrine is used as monotherapy.

Neither AQ nor PPQ caused embryo deaths or malformations in rodents. PYR caused embryo deaths in rabbits, but only at a dose that was excessively toxic to the dams.

# 5 Safety of quinine and artemisinin compounds in the first trimester of human pregnancy

**Because of concern about the safety of ACTs, until recently QNN plus clindamycin had been the recommended medicine for treatment of uncomplicated *P. falciparum* malaria in women in the first trimester of pregnancy.**

**Nevertheless, women are frequently exposed to ACTs in early pregnancy, because they are unaware that they are pregnant; QNN is not available; or health care workers and dispensers in drug outlets have poor knowledge of, or adherence to, national treatment guidelines for the management of malaria in the first trimester *(53, 54, 122, 123)*. This section summarizes the available evidence on the risks of exposure to QNN and artemisinin derivatives in the first trimester of pregnancy.**

**This review is partly based on a series of materials prepared for the meeting of the WHO evidence review group on malaria in pregnancy held in Geneva on 13–16 July 2015, as well as contributions made by that group, updated with new evidence presented to the Guideline Development Group in April 2022 and relevant publication *(57)*. The literature review method is described in the published paper *(57)*. The literature search encompassed any studies published up to December 2021. The method of the literature review for the systematic review and meta-analysis is provided under section 4.1.7.**

## 5.1 Comparative profiles of quinine and ACTs

QNN is a cinchona alkaloid, available in the form of various salts, of which the most widely used are the dihydrochloride and the sulfate *(124)*. It is used as a schizonticidal against intra-erythrocytic malaria parasites and as a gametocytocidal against *P. vivax* and *P. malariae*. QNN is one of the oldest antimalarials – it has been used for almost 400 years *(125)*. However, its mechanism of action is unknown.

Artemisinin compounds are effective against all *Plasmodium* species, and ACTs are currently recommended as the first-line treatment for uncomplicated *P. falciparum* malaria; injectable AS is recommended for severe malaria in children. ACTs may also be used to treat uncomplicated *P. vivax*, *P. ovale*, *P. knowlesi* and *P. malariae* malaria, and are the recommended treatment in areas of chloroquine-resistant *P. vivax* infections. ACTs are combinations of a fast-acting artemisinin derivative with a slower-acting, structurally unrelated antimalarial, resulting in a very high parasite reduction ratio of about 10 000 times per cycle. ACTs administered over 3 days affect two asexual cycles, resulting in a reduction of parasites by approximately 100 million *(126)*. Artemisinins affect the proportion of mosquitoes infected by treated individuals, which may reduce overall malaria transmission *(127, 128)*. The ACTs recommended by WHO for the treatment of uncomplicated malaria are AL, AS-AQ, AS-MQ, AS-SP, DHA-PPQ and, most recently, AS-PYR *(5)*. ACTs are recommended by WHO for treatment of malaria in the second and third trimester of pregnancy, and recently WHO released a strong recommendation for using artemether–lumefantrine in the first trimester of pregnancy *(5)*. All ACTs are available as fixed-dose combinations, except for AS-SP.

The mechanism of the antimalarial effect of artemisinins is being investigated. In a chemical proteomic study, proteins with a variety of biological functions that are alkylated by artemisinins were identified *(129)*. These proteins are involved in pathways

such as glycolysis and haemoglobin digestion, DNA synthesis, protein synthesis and lipid synthesis, all of which are essential for parasite survival, suggesting that artemisinins have a pleiotropic mechanism of action as antimalarial medicines.

### 5.1.1 Pharmacokinetics

QNN is rapidly absorbed after either oral or parenteral administration, reaching peak concentrations within 1–3 hours *(130)*, with a half-life of 11–18 hours *(131, 132)*. Therefore, QNN confers limited post-treatment prophylactic effect. For parasite clearance, QNN is administered every 8 hours for 7 days. QNN readily crosses the placental barrier and can be found in cerebrospinal fluid. About 80% of the administered medicine is eliminated by hepatic biotransformation, and the remaining 20% is excreted unchanged by the kidney *(133)*.

The pharmacokinetics of QNN depend on age (smaller volume of distribution in young children than in adults, slower elimination in the elderly) and the severity of malaria (in acute malaria, the volume of distribution is reduced, and systemic clearance is slower, resulting in higher plasma QNN levels).

Artemisinins are metabolized rapidly in vivo to DHA, which has a bioavailability of >60% and a peak concentration usually achieved within 4 hours *(126)*. They are rapidly eliminated by metabolic biotransformation and have a half-life of approximately 1 hour. The non-artemisinin partner drugs in ACTs lead to longer treatment half-lives than treatment with QNN and, therefore, confer post-treatment prophylaxis of several weeks *(134, 135)*.

The pharmacokinetics of some medicines administered orally, including antimalarials, may be altered in pregnancy because of factors such as the increase in the distribution volume; increased or decreased clearance; changes in protein binding, lipid distribution and absorption of medicines; and hormonal changes *(136)*. Such physiological changes in pregnancy may alter drug exposure; lower plasma concentrations of antimalarials have been found in pregnant than in non-pregnant women with malaria after oral administration of chloroquine *(137, 138)*, lumefantrine *(139, 140)*, MQ *(141)*, proguanil *(142–144)*, atovaquone *(142)*, AS and DHA *(145–147)*, although other studies of these antimalarials showed no significant effect on pharmacokinetics in pregnancy. The discrepancy may be explained by differences in study design, whether with self-matched postpartum or prospectively matched non-pregnant women or historical controls, and the small samples in most studies. Although the results have been inconsistent, decreased exposure to antimalarial medicines during pregnancy could increase the risk of treatment failure, shorten the post-treatment prophylactic period and increase the risk of resistance, whereas increased exposure could lead to toxicity and safety issues. Thus, the response of pregnant women to treatment should be closely monitored, and dose optimization studies should be conducted in this at-risk population. Despite known differences in exposures of some compounds in pregnant women, drug labels do not include specific dose adjustment.

### 5.1.2 Tolerability

QNN (reviewed in *(124)*) has a low therapeutic index and adverse effects. These include mild effects such as tinnitus, slight impairment of hearing, headache and nausea, and more severe effects such as vertigo, vomiting, abdominal pain, diarrhoea, marked auditory loss and visual symptoms, including loss of vision. Hypotension and arrhythmia may occur if the medicine is given too rapidly, and venous thrombosis has been reported after intravenous injection *(133)*.

Intramuscular administration is painful and may cause sterile abscesses. Hypoglycaemia is a side-effect, particularly in pregnant women *(148)*. Other serious but less frequent side-effects include skin eruptions, asthma, thrombocytopenia,

hepatic injury and psychosis *(4)*. Serious side-effects may include cytopenia and haemolytic–uraemic syndrome. QNN is poorly tolerated and is given over a long period, and these two factors may result in poor adherence to therapy *(124)*.

Generally, ACTs are well tolerated and the adverse events are mild, with prompt recovery *(149–151)*. The main adverse events reported in clinical trials are headache, musculoskeletal pain, fatigue, dizziness, abdominal pain, anorexia, nausea and vomiting. These effects are usually mild and resolve spontaneously. Serious adverse events are uncommon. Type 1 hypersensitivity reactions, ranging from mild skin rashes to life-threatening anaphylactic shock, have been reported among non-pregnant adults. The frequency of these events is unknown (reported to range from approximately 1/1000 to 1/3000) *(152)*. Elevated liver enzymes, electrocardiogram abnormalities and rare cases of delayed haemolysis have also been reported *(153–155)*.

The artemisinin present in ACTs is well tolerated, and the partner medicine may be responsible for most of the observed adverse effects *(126)*, which are usually mild and self-limiting. MQ commonly induces nausea, vomiting, dysphoria and dizziness. It is the only partner medicine that can cause frequent, dose-dependent, mild to moderate adverse effects, particularly gastrointestinal adverse effects and dizziness. It can also have more serious effects, such as a self-limiting acute neuropsychiatric syndrome manifesting as encephalopathy, convulsions or psychosis, and suicide *(156)*. The risk for this acute neuropsychiatric syndrome is greater in patients with a previous history of psychiatric illness or epilepsy, or when the medicine is used after severe malaria or was used in the previous 2 months. Concern about the safety of MQ was addressed in a study of patients at the Thailand–Myanmar–Cambodia border *(157)*. Neuropsychiatric side-effects associated with MQ use were rare (11.9 and 7.8 per 10 000 treatments at doses of 25 and 15 mg/kg, respectively). MQ tolerability did not improve when a 15 mg/kg dose administration was split over 2 days in a multicentre randomized controlled trial evaluating MQ for intermittent preventive treatment in women in Benin, Gabon, Mozambique and the United Republic of Tanzania *(158)*. A Cochrane review on the use of MQ for preventing malaria in pregnancy concluded that the high prevalence of drug-related adverse events constitutes an important barrier to its effectiveness for preventive treatment *(159)*.

The only comparison of the safety of different ACTs in pregnancy is from an open-label trial among 3428 pregnant women in the second or third trimester with *P. falciparum* malaria in four African countries, who were treated with one of four ACTs: AL, AS-AQ, AS-MQ or DHA-PPQ. There was no significant difference in the rate of serious adverse events or birth outcomes among the treatment groups *(135)*. Drug-related adverse events, such as asthenia, poor appetite, dizziness, nausea and vomiting, occurred significantly more frequently in the groups given AS-MQ (50.6%) or AS-AQ (48.5%) than in those given DHA-PPQ (20.6%) or AL (11.5%). The following serious drug-related adverse events were reported: anaemia (AS-AQ, AS-MQ), gastrointestinal effects (AL, AS-AQ), malaise (AL, AS-AQ, AS-MQ), and headache and general weakness (DHA-PPQ).

The adverse effects of QNN and ACTs used for uncomplicated *P. falciparum* malaria have been compared in 14 controlled trials with a total of 1996 cases in Bangladesh, Brazil, Cambodia, Gabon and Thailand. A meta-analysis of the results of these trials indicated that the risk of tinnitus was significantly higher with QNN plus antibiotics than with ACTs *(160)*. A meta-analysis of clinical trials among second- and third-trimester pregnancies showed that AL had the best tolerability profile compared with other ACTs (AS-AQ, AS-MQ, DHA-PPQ and AS-SP). This meta-analysis also reported that ACTs were associated with less tinnitus (pooled relative risk [PRR] 0.19; 95% CI: 0.03–1.11), dizziness (PRR 0.64; 95% CI: 0.44–0.93) and vomiting (PRR 0.33; 95% CI: 0.15–0.73) than QNN *(161)*. In one of the studies, a significantly higher proportion of women given QNN than those given ACT had hypoglycaemia. This was confirmed in a recent meta-analysis that showed that, compared with AL, QNN was associated with a higher risk of tinnitus (adjusted odds ratio [aOR] 249.84; 95% CI: 80.90–771.56), abdominal pain (aOR 1.81; 95% CI: 1.07–3.04), anorexia (aOR 4.29; 95% CI: 1.99–9.23), dizziness (aOR 10.25; 95% CI: 6.08–17.28), fatigue (aOR 2.43; 95% CI: 1.02–5.78), vomiting (aOR 9.61; 95% CI: 4.70–19.63) and nausea (aOR 7.29; 95% CI: 3.78–14.08) *(162)*.

### 5.1.3 Drug interactions

When QNN or artemisinins are administered with other medicines, interactions can result from induction or inhibition by the other medicine of enzymes involved in the metabolism of the antimalarial, leading to reduced or increased plasma concentration, due to faster or slower clearance, respectively. Although reports of the effects of nevirapine-based antiretroviral therapy (ART) on exposure to ACTs have been inconsistent, concomitant treatment with efavirenz-based ART generally resulted in significantly lower exposure to AL and DHA-PPQ *(163–173)*. In addition, concomitant administration of efavirenz-based ART was associated with hepatotoxicity *(174)*. The new WHO guidelines for malaria recommend avoiding concomitant use of AS-AQ in patients taking zidovudine, efavirenz and co-trimoxazole (unless AS-AQ is the only ACT promptly available). This recommendation is based on the associated neutropenia in HIV-coinfected patients (especially those on zidovudine and/or co-trimoxazole) and the increased exposure to AQ and hepatotoxicity in patients on efavirenz *(5)*. A clinical trial assessing MQ for intermittent preventive treatment of malaria in pregnancy in HIV-positive women reported a reduced nevirapine concentration – this could explain the observed two-fold increased risk of mother-to-child transmission of HIV among infants born to MQ recipients compared with the placebo-exposed infants *(175)*.

ARTs that include protease inhibitors generally increase the plasma level of lumefantrine by inhibiting enzymes, increasing the risk of toxicity from lumefantrine. In a small study of HIV patients in South Africa given AL with lopinavir- or ritonavir-based ART, the lumefantrine concentration had increased 10-fold by day 7; however, detailed investigations for clinical, haematological, biochemical or electrocardiographic adverse events showed no safety concern *(176)*. The authors suggested that the effect of elevated lumefantrine concentrations on adults with both malaria and HIV infection should be further evaluated to determine whether they reduce the risk of treatment failure, as has been reported in malaria patients and children with both malaria and HIV infection. Interactions between antimalarials such as QNN, artemisinin and its derivatives, and ACT medicines were summarized by Koleba et al. at the Toronto General Hospital in Canada in 2014 *(177)*.

Nevirapine, for example, can interact with QNN when administered concomitantly *(178, 179)*, as exposure to QNN is likely to be decreased by induction of CYP3A4. Surprisingly, concomitant administration of lopinavir–ritonavir also decreased exposure to QNN and its major active metabolite (3-hydroxyquinine) in healthy volunteers *(180, 181)*. The QNN concentration may also be decreased when QNN is taken with rifampicin because of increased clearance due to induction of CYP3A4. This leads to high treatment failure rates – the likelihood of malaria recrudescence is 5 times higher than with QNN alone – and this combination is therefore not recommended *(182)*.

Although no studies of drug–drug interactions have been performed in pregnant women, enzyme induction may exacerbate the lowered exposure that is observed with some antimalarials during pregnancy. Drug–drug interactions can therefore potentially increase the risk of treatment failure and lead to a shorter post-treatment prophylactic period. The response of pregnant women to treatment should thus be closely monitored, and dose optimization studies should be performed for these doubly vulnerable populations. Physiologically based pharmacokinetic modelling may also be used as a tool to support dose optimization in pregnant women, especially when taking medications for multiple indications *(183)*. Efavirenz- and rifampicin-based treatment should not be stopped during antimalarial treatment, as their induction effects would persist during and beyond the duration of malaria *(184, 185)*. However, AS-AQ and antimalarials that are CYP3A4 substrates should not be administered to patients taking efavirenz-based ART *(5)*. Dolutegravir-based ART is least likely to be involved in drug–drug interactions.

### 5.1.4 Efficacy

No data are available on the efficacy of malaria treatment in the first trimester of pregnancy; the only available data are from studies in the second and third trimesters. A meta-analysis of six randomized controlled trials in sub-Saharan Africa and Thailand was conducted to compare the efficacy, safety and tolerability of ACTs versus QNN for the case management of uncomplicated *P. falciparum* malaria in the second and third trimesters *(161)*. ACTs were better tolerated and much more effective than oral QNN and were associated with faster parasite clearance, lower polymerase chain reaction (PCR)–corrected treatment failures and mean birth weights that were higher by 75 g. A more recent meta-analysis reported that the risk of treatment failure was 6 times higher for QNN than AL treatment in pregnancy (aHR 6.11; 95% CI: 2.57–14.54) *(162)*. Both meta-analyses reported all ACTs to have PCR-adjusted cure rates >90% in the second and third trimesters. A multicentre, randomized, open-label trial of the four co-formulated ACTs was conducted with 3428 pregnant women with *P. falciparum* malaria in the second or third trimester of pregnancy in four African countries to evaluate the efficacy of four ACTs: AL, AS-AQ, AS-MQ and DHA-PPQ *(135)*. All four ACTs effectively cleared existing infections but differed in their ability to prevent new infections. The unadjusted cure rate was significantly lower with AL (52.5%) than with the other ACTs (>70%) because of the shorter half-life of lumefantrine. DHA-PPQ was the most efficacious.

### 5.1.5 Documented exposure to artemisinins in the first trimester

Because of ethical and methodological constraints, limited information has been published on human exposure to artemisinin derivatives in the first trimester since the introduction of ACTs 22 years ago. By December 2021, 1340 documented first-trimester exposures had been reported from published and unpublished studies (Table 2). Except for a study in Indonesia *(186)*, which reported confounding by indication, none of the studies found an association between first-trimester exposure to artemisinins and adverse pregnancy outcomes, although not all the studies included a comparison group. Although this overall conclusion is reassuring, the studies have important limitations, including the observational and (in some cases) retrospective nature of their design *(187, 188)*, late enrolment that precluded identification of early miscarriage, and lack of assessment of cardiovascular and other specific congenital anomalies. About half of the exposures ($n$ = 575) were to AL.

**Table 2. Number of confirmed first-trimester exposed pregnancies for each artemisinin treatment type, listed by publication year**

| Author | Country | Publication year | Number of confirmed first-trimester exposures | AL | DHA-PPQ | AS-AQ | AS-MQ | AAP | AS-SP | AS-PYR | AS | AS (IV/IM) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **McGready *(21, 189)*** | Thai–Myanmar border | Published and unpublished, 2000–2020 | 351 | 28 | 28 | 0 | 65 | 3 | | | 228 | 10 |
| **Deen *(187)*** | Gambia | 2001 | 77 | | | | | | 77 | | | |
| **Adam *(190)*** | Sudan | 2004 | 1 | | | | | | | | | 1 |
| **Adam *(191)*** | Sudan | 2009 | 62 | 3 | | | | | 11 | | | 48 |
| **Manyando *(192)*** | Zambia | 2010 | 156 | 156 | | | | | | | | |
| **Mehta *(47)*** | Kenya, Ghana, Uganda, United Republic of Tanzania | 2012 | 15 | 10 | 2 | 2 | | | | | | 1 |
| **Rulisa *(193)*** | Rwanda | 2012 | 96 | 96 | | | | | | | | |
| **Dellicour *(194)*** | Senegal | 2013 | 7 | | | 7 | | | | | | |
| **Mosha *(122)*** | United Republic of Tanzania | 2014 | 168 | 168 | | | | | | | | |
| **Poespoprodjo *(186)*** | Indonesia | 2014 | 18 | | 13 | | | | | | | 10 |
| **Dellicour *(48, 195)*** | Kenya | 2015 | 85 | 85 | | | | | | | | |
| **Sevene *(48)*** | Mozambique | 2015 | 21 | 21 | | | | | | | | |
| **Tinto *(48)*** | Burkina Faso | 2015 | 41 | 1 | | 40 | | | | | | |
| **Rouamba *(196)*** | Burkina Faso | 2018 | 19 | 7 | 5 | 5 | | | | | 2 | |
| **Ahmed *(188)*** | Indonesia | 2019 | 204 | | 204 | | | | | | | |
| **Rouamba *(197)*** | Burkina Faso | 2020 | 13 | | | 13 | | | | | | |
| **Lutete *(198)*** | Democratic Republic of the Congo | 2021 | 6 | | | | | | | 6 | | |
| **Total** | **13** | **2000–2022** | **1340** | **575** | **252** | **67** | **65** | **3** | **88** | **6** | **230** | **70** |

AAP: artesunate–atovaquone–proguanil; AL: artemether–lumefantrine; AS: artesunate; AS-AQ: artesunate–amodiaquine; AS-MQ: artesunate–mefloquine; AS-PYR: artesunate–pyronaridine; AS-SP: artesunate–sulfadoxine–pyrimethamine; DHA-PPQ: dihydroartemisinin–piperaquine; IM: intramuscular; IV: intravenous.

### 5.1.6 Assessment of risk of adverse pregnancy outcomes for ABT versus non-ABT

A recent updated individual-patient meta-analysis of seven prospective cohort studies found no increase in the risk of adverse pregnancy outcomes (miscarriage, stillbirth or major congenital anomalies) associated with exposure to artemisinin-based treatment (ABT) early in pregnancy compared with non-artemisinin-based treatment (non-ABT) for malaria *(57)*. Furthermore, first-trimester treatment with AL was associated with significantly fewer (42% lower) adverse pregnancy outcomes than first-trimester treatment with QNN.

This meta-analysis included all prospective cohort studies up to December 2021 that enrolled women before the outcome of pregnancy was known, contained information on first-trimester exposures to artemisinins or other antimalarial treatment, and included both women exposed to ABT and their comparators (either women exposed to non-ABT or women unexposed to any antimalarials). Women were included regardless of parasitological confirmation or species. Fourteen studies were identified, of which seven were eligible, and all were included in the individual-patient analysis: six from sub-Saharan Africa *(47, 48, 122, 192, 193, 196)* and one from the Shoklo Malaria Research Unit in Thailand *(21, 188)*. Data on individual participants used in the meta-analysis were obtained from all eligible cohort studies. Exposure to antimalarials was ascertained by self-reporting or active detection, and confirmed using clinic cards and outpatient registers. The primary outcome was adverse pregnancy outcome, defined as the composite of a pregnancy ending in either miscarriage (spontaneous pregnancy loss <28 weeks), stillbirth (pregnancy loss ≥28 weeks) or major congenital anomalies (any structural abnormality with surgical, medical or cosmetic importance that is present at birth, detected by surface examination, excluding cases of genetic etiology). Hazard ratios were obtained from Cox regression models with shared frailty accounting for within-cohort clustering through one-stage meta-analysis of individual-participant data. Overall, 34 178 pregnancies met the inclusion criteria, including 737 with confirmed first-trimester exposure to ABT and 1076 with confirmed exposure to non-ABT. There were no differences between pregnancies exposed in the first trimester to artemisinins versus non-artemisinins in the composite adverse pregnancy outcome (ABT: 42/736 [5.7%]; non-ABT: 96/1074 [8.9%]; aHR 0.71; 95% CI: 0.49–1.03), miscarriage (ABT: 27/669 [4.0%]; non-ABT: 76/1070 [7.1%]; aHR 0.74; 95% CI: 0.47–1.17), stillbirth (ABT: 13/646 [2.0%]; non-ABT: 12/743 [1.6%]; aHR 0.71; 95% CI: 0.32–1.57) or major congenital anomalies (ABT: 2/736 [0.3%]; non-ABT: 8/1074 [0.7%]; aHR 0.60; 95% CI: 0.13–2.87) (Table 3). There was also no difference in the risk of these adverse pregnancy outcomes when exposures were restricted to the putative embryo-sensitive period.

**Table 3. aHR and 95% CIs for adverse pregnancy outcomes (miscarriage, stillbirth or major congenital anomalies) associated with exposure to artemisinins or to non-artemisinin antimalarial treatment in early human pregnancy**

| Period | ABT (no. events/ total) | Non-ABT (no. events/total) | aHR (95% CI) | *P* |
|---|---|---|---|---|
| Composite adverse pregnancy outcome | | | | |
| **First trimester (2–13 weeks post-LMP)** | 42/736 | 96/1074 | 0.71 (0.49–1.03) | 0.071 |
| **Putative embryo-sensitive period (6–12 weeks post-LMP)** | 37/584 | 60/822 | 0.95 (0.63–1.45) | 0.828 |
| Miscarriage | | | | |
| **First trimester (2–13 weeks post-LMP)** | 27/669 | 76/1070 | 0.74 (0.47–1.17) | 0.195 |
| **Putative embryo-sensitive period (6–12 weeks post-LMP)** | 23/533 | 46/818 | 1.02 (0.61–1.70) | 0.95 |
| Stillbirth | | | | |
| **First trimester (2–13 weeks post-LMP)** | 13/646 | 12/743 | 0.71 (0.32–1.57) | 0.395 |
| **Putative embryo-sensitive period (6–12 weeks post-LMP)** | 12/518 | 6/608 | 1.18 (0.44–3.18) | 0.746 |
| Major congenital anomalies | | | | |
| **First trimester (2–13 weeks post-LMP)** | 2/736 | 8/1074 | 0.60 (0.13–2.87) | 0.521 |
| **Putative embryo-sensitive period (6–12 weeks post-LMP)** | 2/584 | 8/822 | 0.72 (0.15–3.49) | 0.688 |

ABT: artemisinin-based treatment; aHR: hazard ratio; CI: confidence interval; LMP: last menstrual period; non-ABT: non-artemisinin-based antimalarial treatment.

*Notes:* aHRs account for pregnancy week under observation through left-truncation, with exposure as a time-dependent variable, and are adjusted for maternal age, gravidity and calendar year. Estimates were derived in a random-effect individual data meta-analysis using a shared-frailty model to account for within-study clustering. The numbers in the ABT and non-ABT columns represent the pregnancies included in the adjusted analysis, which excludes three women (one exposed to AL and two exposed to QNN) with a missing covariate (gravidity).

*Source:* Saito et al. *(57).*

A comparison with women who were not exposed to antimalarials in the first trimester of pregnancy suggested a significantly increased risk of adverse pregnancy outcomes associated with non-ABT but not with ABT (aHR 1.30; 95% CI: 1.06–1.60; and aHR 0.92; 95% CI: 0.67–1.26, respectively). However, this comparison with women not exposed to antimalarial agents during the first trimester of pregnancy was a secondary analysis because of the probability of confounding by indication.

Overall, the data rule out a 45% (aHR of 1.45) increase in the risk of adverse pregnancy outcomes with artemisinin treatment compared with non-artemisinin-containing treatments, as suggested by the upper confidence limit of the most conservative estimate for the suggested embryo-sensitive period for artemisinins. The data on miscarriage rule out an increase in risk greater than 1.70 times. Similarly, for stillbirth, the upper confidence limit of the hazard ratio rules out an increase in the risk of >3.18 times. The individual-patient meta-analysis showed no difference in the prevalence of major congenital anomalies between exposure in the first trimester to artemisinins compared with QNN (ABT: 2/736 [0.3%]; non-ABT: 8/1074 [0.7%]; aHR 0.60; 95% CI: 0.13–2.87) or non-exposure (ABT: 2/736 [0.3%]; unexposed: 182/32203 [0.6%]; aHR 0.99; 95% CI: 0.24–4.03). The congenital anomalies detected in the artemisinins-exposed group in early pregnancy included a case of bilateral syndactyly, a case of imperforated anus, and a case of cleft lip and palate. None of these were signals identified in the animal studies. The eight non-ABT in utero exposure cases of congenital anomalies in the first trimester and embryo-sensitive period were two cases of cleft lip and palate, two cases of unilateral clubfoot, one case of syndactyly (both hands and feet) and bilateral clubfoot, one case of congenital heart defect, one case of polydactyly and missing toe, and one case of bilateral brachy-syndactyly. No congenital heart defects, reported in animals, were observed in artemisinin-exposed pregnancies, although cardiac auscultation of newborns was systematically assessed only in one study, and other studies did not systematically screen for heart defects.

#### 5.1.7 Assessment of risk of adverse pregnancy outcomes for artemether–lumefantrine versus quinine

Most documented treatment with ACT in the first trimester was with AL. An analysis restricted to a comparison between AL and oral QNN exposure in the first trimester suggested a 42% lower risk with AL (AL: 25/524 [4.8%]; QNN: 84/915 [9.2%]; aHR 0.58; 95% CI: 0.36–0.92) for the primary composite end-point. This was 29% lower (non-significant) for exposures during the putative embryo-sensitive period (AL: 22/445 [4.9%]; QNN: 51/684 [7.5%]; aHR 0.71; 95% CI: 0.43–1.20) (Table 4). The numbers were too small to conduct further analyses for the other ACTs.

No major congenital anomalies were detected in the 482 live births from pregnancies exposed to AL in the first trimester (0/482; 95% CI: 0–0.79%). This upper confidence limit is similar to the 0.69% background rate of major congenital anomalies detected at birth by surface examination in the group unexposed to antimalarials (182/26 270; 95% CI: 0.60–0.80) and the 0.74% in the QNN-exposed group (4/545; 95% CI: 0.29–1.88).

**Table 4. aHRs and 95% CIs for risks of adverse pregnancy outcomes (miscarriage, stillbirth or major congenital anomalies) associated with exposure to AL compared with oral QNN in early human pregnancy**

| Period | AL (no. events/ total) | QNN (no. events/total) | aHR (95% CI) | *P* |
|---|---|---|---|---|
| Composite adverse pregnancy outcome | | | | |
| **First trimester (2–13 weeks post-LMP)** | 25/524 | 84/915 | 0.58 (0.36–0.92) | 0.021 |
| **Putative embryo-sensitive period (6–12 weeks post-LMP)** | 22/445 | 51/684 | 0.71 (0.43–1.20) | 0.200 |
| Miscarriage | | | | |
| **First trimester (2–13 weeks post-LMP)** | 15/464 | 68/913 | 0.67 (0.37–1.23) | 0.196 |
| **Putative embryo-sensitive period (6–12 weeks post-LMP)** | 12/398 | 40/682 | 0.77 (0.39–1.52) | 0.445 |
| Stillbirth | | | | |
| **First trimester (2–13 weeks post-LMP)** | 10/488 | 12/590 | 0.53 (0.22–1.24) | 0.142 |
| **Putative embryo-sensitive period (6–12 weeks post-LMP)** | 10/415 | 6/469 | 0.90 (0.32–2.51) | 0.841 |
| Major congenital anomalies | | | | |
| **First trimester (2–13 weeks post-LMP)** | 0/524 | 4/915 | NA | NA |
| **Putative embryo-sensitive period (6–12 weeks post-LMP)** | 0/445 | 5/684 | NA | NA |

aHR: adjusted hazard ratio; AL: artemether–lumefantrine; CI: confidence interval; LMP: last menstrual period; NA: not applicable; QNN: quinine.

*Notes:* aHRs account for pregnancy week under observation through left-truncation, with exposure as a time-dependent variable, and are adjusted for maternal age, gravidity and calendar year. Estimates were derived in a random-effect individual data meta-analysis using a shared-frailty model to account for within-study clustering. The numbers in the AL and QNN columns represent the pregnancies included in the adjusted analysis, which excludes three women (one exposed to AL and two exposed to QNN) with a missing covariate (gravidity).

The safety, tolerability, efficacy, ease of use and drug interactions of QNN and ACT are summarized in Table 5.

**Table 5. Clinical characteristics of QNN and ACT**

| Characteristic | QNN | ACT |
|---|---|---|
| **Safety and tolerability** | Recommended for use in pregnancy, although most of the evidence is old (no trials have been conducted on first-trimester exposure).<br><br>Poorly tolerated, but most adverse events are mild and resolve relatively quickly. Serious adverse events are rare; they include skin eruption, asthma, thrombocytopenia, hepatic injury, psychosis, cytopenia and haemolytic–uraemic syndrome; hypoglycaemia is a side-effect particularly in pregnant women. | No evidence of harm documented in >1000 human exposures.<br><br>Well tolerated; mild adverse events and rare severe events. In a trial in patients in the second and third trimesters of pregnancy who had *P. falciparum* malaria, asthenia, poor appetite, dizziness, nausea and vomiting occurred significantly more frequently in the group given AS-MQ (50.6%) or AS-AQ (48.5%) than in those given DHA-PPQ (20.6%) or AL (11.5%). |
| | No difference in the risk of adverse pregnancy outcomes (stillbirth, miscarriage or major external congenital anomalies) between pregnancies treated with an ACT or QNN in the first trimester. ACTs are well tolerated, whereas QNN is poorly tolerated (higher prevalence of dizziness, vomiting and hypoglycaemia). | |
| **Efficacy** | No data on efficacy in the first trimester of pregnancy have been reported; however, both QNN and ACTs are effective. Two meta-analyses of randomized controlled trials from sub-Saharan Africa and Asia of women with uncomplicated *P. falciparum* malaria in the second and third trimesters of pregnancy showed that ACTs were more effective than oral QNN, with faster parasite clearance, lower PCR-corrected treatment failure and higher mean birth weights. | |
| **Ease of use** | Available in oral and parenteral formulations, administered every 8 hours for 7 days. | Available as fixed-dose co-formulations[2] of an artemisinin derivative and a longer-acting partner medicine of a different class; WHO recommends that ACTs be given for 3 days once or twice a day. |
| **Drug interactions** | Potentially decreased plasma concentration (exposure) when co-administered with antiretrovirals including efavirenz and rifampicin. Response to treatment should be monitored closely. | |

## 5.2 Possible reasons for embryotoxicity observed in animal studies not being observed in humans

### 5.2.1 Quinine

Despite the findings from rodent studies that demonstrated embryotoxic effects of QNN, and a few human reports associating first-trimester in utero exposure to QNN with inner ear abnormalities, this compound has remained the recommended therapy for uncomplicated malaria during the initial trimester for more than 2 decades. This recommendation has been grounded in historical precedent, as no

2 With the exception of artesunate plus sulfadoxine-pyrimethmine, available as co-blister only and not recommended for use in the 1st trimester of pregnancy.

randomized treatment trials with QNN in the first trimester have been conducted. Observational data encompassing treatment of 900 pregnant women with malaria in the first trimester with QNN imply that the risk of congenital anomalies is not notably heightened. It has been suggested that malaria protects against the developmentally toxic effects of QNN, perhaps by reducing the placental transfer of QNN *(97)*; however, this hypothesis has not been validated in animal or human studies.

### 5.2.2 Artemisinins

Sensitivity to artemisinins' effects in human fetuses would probably have been detected by now if humans were as susceptible as rats and monkeys. The evidence from the updated meta-analysis excludes an increased risk of overall major congenital anomalies of 3.5-fold and pregnancy loss (miscarriage or stillbirth) of 1.5-fold for pregnant women treated with an artemisinin during the putative sensitive period (6–12 weeks from last menstrual period when primitive erythroblasts are in circulation) *(57)*. In vitro studies have revealed that DHA exerts dose- and time-dependent effects on human erythropoiesis *(199)*. Notably, its toxicity is confined to primitive erythropoietic stages, reflecting the susceptibility observed in rats and monkeys. The identification of analogous target cells in human cultured systems raised concerns regarding potential embryotoxic sensitivity in humans. However, available comprehensive human pregnancy data for specific artemisinins suggest this assumption may not hold true. This lack of embryotoxicity observed in humans prompts consideration of conceivable explanations, which are elaborated upon below.

1. The length and dosage of exposure play an important role in the embryotoxicity of artemisinins. In mice and rats, the primary target of embryotoxicity, nucleated primitive erythroblasts, are produced over only a few days. In mice (and presumably rats), haemopoiesis occurs in the visceral yolk sac only about 2 days before the progenitor-derived cells are transferred to the blood to form nucleated primitive erythroblasts, which continue to divide in the circulation *(79, 200)*. If these animals are treated with an artemisinin during this period, substantial depletion of primitive erythroblasts can occur. Once erythroblasts are depleted, there is limited ability to replace them, resulting in severe consequences for fetal development.

   It is unknown when the progenitor cells occur in the visceral yolk sac in monkeys. Embryotoxicity in monkeys was observed when artesunate was administered at 12 mg/kg per day for 12 days or longer, but not in animals treated for 3 or 7 days, indicating that treatment courses shorter than 12 days at that dose (HED = 3.9 mg/kg) are unlikely to cause significant depletion of embryonic erythroblasts in this species.

   In humans, haematopoietic progenitors of primitive erythroblasts are found within blood islands in the visceral yolk sac between post-conception weeks 3 and 7 *(201, 202)*. Primitive erythroblasts develop over 6 weeks and are predominant in the circulation of the human embryo between weeks 4 and 10 weeks post-conception *(200)*. Therefore, any transient reductions of circulating primitive erythroblasts caused by short-term exposure to an artemisinin can be replenished by newly produced cells and have no clinically significant effect. In contrast to animal models, a short treatment course of 3–7 days with AS at a target daily dose of 4 mg/kg per day in humans is unlikely to lead to embryotoxicity.

2. Although the embryotoxicity of artemisinin derivatives in animals is postulated to occur through depletion of primitive erythroblasts, the molecular target for artemisinins is not identified yet. The human embryo may be less sensitive to their

effects than the corresponding target in the embryos of other animal species. For example, a possible target that might have a different structure in humans and the animal species tested is ferrochelatase, the enzyme that mediates the last step in haem biosynthesis. The amino acid sequence of ferrochelatase is known for three mammals (humans, cattle and mice), and all differ *(203–206)*. Confirmation of this hypothesis will be contingent upon the identification of the molecular target of artemisinins.

3. The relative sensitivity to artemisinins of human embryos compared with other species is unknown. Human cells undergoing erythropoiesis have been shown to be sensitive to artemisinins. An in vitro study found that 2 μM of DHA inhibited proliferation and differentiation of CD34+ cells derived from the peripheral blood of adult humans and induced them to undergo erythropoiesis in cell culture *(199)*. Determining whether therapeutic doses of artemisinins lead to a blood concentration of 2 μM DHA in human embryos remains an elusive challenge. It is conceivable that other species undergoing erythropoiesis exhibit heightened sensitivity to artemisinins at considerably lower concentrations than humans, potentially accounting for the observed variations.

4. It has been hypothesized that malaria may protect against embryotoxicity, by sequestering artemisinins in infected red blood cells and reducing the amount that would cross the placenta and reach the embryo *(82, 207, 208)*. This could be a potential explanation, as the animal studies were conducted in healthy animals, whereas clinical studies of artemisinins in pregnant women have been conducted as part of management of patients with malaria symptoms, although malaria diagnosis information was unavailable for some of the observational studies and some cases may have been treated presumptively.

   Artemisinin-induced reticulocytopenia is much more pronounced in uninfected people than in people with malaria *(82)*. If infection with malaria does protect against embryotoxicity, the protection could result from (i) malaria-induced hypoferraemia that diminishes the reactivity of artemisinins in tissues; (ii) accumulation of artemisinin in infected circulating red cells *(209)*, which could reduce the amount of active drug available to cross the placenta; or (iii) binding and accumulation of these malaria-infected erythrocytes in the placenta *(210, 211)*, which could block or retard placental transfer of artemisinins.

   The observation that the volume of distribution of DHA was 2.3 times greater in healthy control subjects given 100 mg AS orally than in patients with malaria *(212)* is consistent with the idea that sequestration of infected red blood cells in the microvasculature interferes with the distribution of DHA to the tissues. This supports the third alternative.

   If this hypothesis is true, malaria-uninfected pregnant women and their unborn babies may be more susceptible to the embryotoxic effects of artemisinins. Nonetheless, it remains unclear whether the proportion of drug retained within infected red blood cells could explain the differences in embryotoxicity observed in animal and human studies. Additional research is warranted to ascertain whether malaria may offer protection against embryotoxicity in humans. The benefit–risk balance for the use of ACTs for chemoprevention in the first trimester has not been evaluated and the current WHO recommendations relate to case management of malaria.

# 6 Safety of non-artemisinin ACT partner medicines in the first trimester of human pregnancy

**This section summarizes the safety of six non-artemisinin ACT partner medicines in the first trimester of human pregnancy, on the basis of the detailed review by Clark *(97)* and other more recent reviews. The medicines are AQ, lumefantrine, MQ, PPQ, SP and PYR.**

## 6.1 Amodiaquine

AQ is considered safe in pregnancy, although there is limited documentation of its safety when used in the first trimester. One observational study in the United Republic of Tanzania included 11 women exposed to AQ early in pregnancy, all of whom had live births with no congenital anomalies *(122)*. A meta-analysis included 32 first-trimester exposures to AS-AQ from three studies in sub-Saharan Africa *(57)*. Although the numbers were too small to conduct a subgroup analysis, the overall analysis of all artemisinin treatments did not show an increase in the risk of adverse pregnancy outcomes over that of women treated with QNN in the same period of pregnancy. Two other studies in Burkina Faso and Senegal not included in the meta-analysis documented an additional 20 women exposed during the first trimester to AS-AQ *(194, 197)*. No adverse pregnancy outcomes associated with these exposures were reported.

## 6.2 Lumefantrine

Lumefantrine is available only in combination with artemether; thus, no information is available on exposure to lumefantrine alone. The effects in more than 500 women exposed to AL in the first trimester have been documented *(57, 213)*. In one study, a higher proportion of miscarriages was found among women exposed to AL in the first trimester (3.8% overall; 4/150 AL only; 2/9 AL + SP) than among women treated with SP and/or QNN (0/135) *(192)*. However, the data were only descriptive, and the analysis of miscarriage did not take into account confounding or other important factors. Overall, the available data do not indicate an increased risk of adverse pregnancy outcomes after first-trimester exposures to AL *(57, 213)*.

## 6.3 Mefloquine

One observational study in Thailand suggested that MQ was associated with an increased risk of stillbirth *(214)*; however, other studies did not confirm this proposal *(135, 215, 216)*. There have been more than 1000 documented first-trimester exposures to MQ *(217)*, and recent reviews of the safety of MQ in pregnancy concluded that there is no evidence that MQ increases the risk of adverse effects on the fetus *(159, 217–219)*.

## 6.4 Piperaquine

No information is available on exposure to PPQ alone, and only limited data are available on exposure to DHA-PPQ in the first trimester of pregnancy ($n$ = 252; see Table 2). In one study in Indonesia, a higher rate of spontaneous abortions was found among women treated with DHA-PPQ (5/8) than among those treated with QNN (1/38) *(186)*. However, the authors noted that confounding by indication was likely, as the women treated with DHA-PPQ were more likely to present with more severe

disease than those treated with QNN. An unpublished retrospective study in Indonesia, where the treatment guidelines have recently been changed to include DHA-PPQ as first-line therapy, including during the first trimester of pregnancy, showed no increase in the risk of pregnancy loss (miscarriage or stillbirth) or major congenital anomalies among 159 pregnant women treated with DHA-PPQ in the first trimester compared with those treated with QNN *(188)*.

## 6.5 Sulfadoxine–pyrimethamine

Sulfadoxine and pyrimethamine are antifolates and are not recommended for use in the first trimester of pregnancy because of the risk of neural tube defects in fetuses *(14, 220, 221)*. Travellers exposed to SP in the first trimester documented in a Roche database did not have higher rates of spontaneous abortion than expected; however, the prevalence of congenital anomalies among infants exposed in utero (7.8%) was higher than expected (e.g. the background rate of malformations in the United States is about 3%). The data are difficult to interpret because there is no appropriate comparison group. There was no consistent pattern in the observed anomalies, and none were neural tube defects *(97)*. In a prospective study in Zambia of 120 women treated with SP in the first trimester of pregnancy *(192)*, there were no spontaneous abortions, and the rate of stillbirth was similar to that of the unexposed group. The prevalence of congenital anomalies among women exposed to SP and/or QNN (data not reported separately; 6.6%) was similar to that in the other groups (6.9% for AL and 4.5% for untreated). No neural tube defects were reported. SP does not appear to be teratogenic in humans; however, information on exposure to SP in the first trimester is limited ($n$ = 300) *(97)*. Although SP is not recommended for use in the first trimester, its safety in the second and third trimesters is supported by extensive clinical trial data, and SP remains the recommended medicine for intermittent preventive treatment in pregnancy.

## 6.6 Pyronaridine

Very limited information is available on PYR treatment in the first trimester of pregnancy. A treatment trial in non-pregnant adults that reported on six accidental first-trimester exposures had 11 pregnancies confirmed during the study *(198)*. Three of these pregnancies were terminated for social reasons, while the remaining eight (six of which were exposed to the study treatment during the first trimester) progressed normally with spontaneous deliveries, except for one case of uterine hypertonus at full term that required caesarean section *(198)*.

The marketing authorization holder of Pyramax® has set up a pregnancy exposure registry to monitor all exposed pregnancies and their outcomes *(222)*. The MiMBa Pregnancy Registry is an ongoing multicentre study in sub-Saharan Africa, collecting observational data on antimalarial exposures early in pregnancy in areas where AS-PYR is used (results anticipated in 2025) *(223)*.

# 7 Conclusions

A total of 1340 first-trimester exposures to ABTs and more than 1000 first-trimester exposures to non-ABTs, including more than 900 exposures to therapeutic doses of QNN (including in combination with clindamycin), have been recorded in human observational studies. Use of artemisinin derivatives in the first trimester was not associated with higher risks of adverse pregnancy outcomes (miscarriage, stillbirth or major congenital anomalies) than use of QNN in seven studies that included a total of 12 268 pregnancies in 11 cohorts in sub-Saharan Africa, and 21 910 pregnant women on the Thailand–Myanmar border who were treated for malaria and followed prospectively. Although all artemisinins tested are embryotoxic in rats, rabbits and monkeys, and the most sensitive embryonic period corresponds to that of humans between post-conception weeks 4 and 10, humans appear to be less sensitive to artemisinins. The lower sensitivity of humans compared with other animals to artemisinins in pregnancy requires further investigations, but could be partly explained by the shorter treatment course and interspecies variation in erythropoiesis, and it has been hypothesized that pregnant women with malaria infections would be at lower risk due to the sequestration of antimalarials in the red blood cells. However, further studies are needed to confirm this latter hypothesis.

Malaria in pregnancy is associated with adverse outcomes for both the mother and the infant. Therefore, both prevention and optimal antimalarial treatment in terms of efficacy, safety and tolerability should be available at this critical period of human development. The benefits of 3-day ACT regimens in treating malaria in early pregnancy should also be considered in the context of the effects on the mother and the fetus of partially treated malaria due to poor adherence to 7-day oral QNN regimens. Additional benefits associated with ACTs include longer post-treatment prophylaxis, potential impact on reduction of onwards malaria transmission, and simplified supply chain management of antimalarials for adult treatments.

In humans, the data to date indicate no evidence of embryotoxicity or teratogenicity resulting in an increased risk of miscarriage, stillbirth or major congenital anomalies associated with ABT during the first trimester of pregnancy or the suggested embryo-sensitive period.

On the basis of this evidence, the WHO recommendation for the treatment of uncomplicated malaria has been updated. It now reads: "Treat pregnant women with uncomplicated *P. falciparum* malaria during the first trimester with artemether–lumefantrine". Monitoring the safety of antimalarial treatment in pregnancy is essential and should be continued, particularly for rare outcomes such as congenital anomalies. Additionally, given the limited data on exposures to other ACTs (AS-AQ, AS-MQ,DHA-PPQ and AS-PYR), WHO recommends: "Continued pharmacovigilance and clinical research, including prospective controlled trials on the efficacy and safety of antimalarial medicines for the treatment of malaria in pregnancy, should be supported and funded".

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