NOT FOR PUBLICATION

COMMISSION ON HUMAN MEDICINES

ADDENDUM: Evaluation of review of transient embryonic hypoxia and teratogenicity in relation to oral hormone pregnancy tests, including Primodos

Type of paper: For Advice

Product(s):	**Assessor(s):**
Oral Hormone Pregnancy Tests, including Primodos	Dr John Clements Dr Janet Nooney
MAHs: Alinter Group Bayer plc GlaxoSmithKline UK Marshall's Pharmaceuticals Ltd Merck, Sharpe and Dohme Ltd Pfizer Piramal Healthcare Ltd Sanofi	
Active constituents(s):	**Previous Assessment:**
Norethisterone acetate; Ethinylestradiol	**HPT EWG 2015-2017** **CHM 2017** **CHM EWG 2018** **CHM EWG 2019**
Therapeutic classification (ATC code):	**Legal status:**
Hormone pregnancy tests	No Longer Marketed

ADDENDUM:
The CHM meeting reference header for the presented paper is CHM/2024/7th, which refers to the July meeting. The original intention was for this paper to be discussed at the July meeting (7th meeting). On May 22nd it was announced there would be an election on July 4th. As a result, and in line with Civil Service policy during a pre-election period, discussion of this paper was postponed until after the election. To note there are no other changes to the paper being considered at the November meeting from the original prepared for the July meeting.

Index

NOT FOR PUBLICATION

Evaluation of review of transient embryonic hypoxia and teratogenicity in relation to oral hormone pregnancy tests, including Primodos

1 ISSUE

An Expert Working Group (EWG) was convened by the Commission on Human Medicines (CHM) in 2015, to review the available data on a possible association between oral hormone pregnancy tests (HPTs) and adverse outcomes in pregnancy. The overall finding of the EWG, endorsed by CHM, was that, taking all aspects into consideration, the available scientific evidence, did not support a causal association between the use of HPTs, such as Primodos, during early pregnancy and adverse outcomes, either with regard to pregnancy loss (miscarriage, stillbirth) or to congenital anomalies.

There has been ongoing interest and parliamentary debate[1] in relation to the findings of the EWG and HPTs were one of the interventions considered in the Independent Medicines and Medical Devices Safety Review (IMMDS review[2], Cumberlege Report). In its response to the IMMDS review, the MHRA gave an undertaking to review relevant new evidence if it emerges.

Moreover, although HPTs have not been available in the UK since 1978, the progestogenic and estrogenic components of HPTs are currently found in a range of widely-used authorised gynaecological medicines across the UK. Any new findings on HPTs could therefore have implications for other hormonal products including oral contraceptives (OCs), treatments for endometriosis, disorders of menstruation, period delay, menopausal hormone replacement therapy and some cancers.

A review by Danielsson, Vargesson and Danielsson published in the journal *Reproductive Toxicology* in December 2023[3], examined the experimental and clinical evidence that hypoxia can lead to formation of reactive oxygen species, which may be teratogenic, following exposure to products with potential to cause failed abortion. The authors include misoprostol and HPTs including Primodos as examples of the latter, with teratogenicity arising secondary to uterine contractions and compression of uterinoplacental/embryonic vessels during organogenesis.

This paper evaluates the Danielsson publication in the context of the data reviewed by the EWG on HPTs and seeks CHM advice on whether more detailed review of this publication by an *ad hoc* EWG is merited at this time.

2 BACKGROUND

2.1 Hormone Pregnancy Tests

A range of oral hormone pregnancy tests (HPTs) were widely used within Europe to diagnose pregnancy from the late 1950s until 1978. Most of this use pre-dated modern licensing requirements and most products were only subsequently formally *licensed* for the treatment of secondary amenorrhoea, rather than for diagnosing pregnancy as such. HPTs contained natural or synthetic sex steroid hormones, usually a progestogen (which mimics the actions of progesterone) in combination with an estrogen. Whilst the roles of

progesterone and estrogens in supporting implantation and early placental development have been defined, their role in fetal organogenesis and development remains less clear.

During the 1950s and 1960s, access to family planning advice and effective contraception was limited and abortion (other than in extreme medical circumstances) was illegal in the UK until 1967. Whilst pregnancy testing became more available from the 1920s onwards, it did not become mainstream or universal until much later. Most women did not usually attend antenatal clinics or consult a doctor about their pregnancy before the second or third trimester. There is some evidence that when HPTs first became available in the UK in the 1950s, testing for pregnancy was intended for women who were considered more at risk of having a complicated pregnancy (Gal 1972[4], Michaelis 1983[5]).

Prior to introduction of HPTs, the alternatives for confirming pregnancy were a physical examination by the doctor or a relatively slow and expensive laboratory test (using toads). At the time of their introduction, and despite questions about their reliability in diagnosing pregnancy, HPTs were therefore considered to have several advantages over the alternatives and recognised as offering a more accessible, quicker and cheaper method of diagnosing pregnancy than the alternatives.

Exact usage data are not known but one source (Gal 1978[6]) has estimated that almost 8 million women in the UK were prescribed an HPT, of which about a million prescriptions were for diagnosing pregnancy.

2.2 Primodos

The most frequently used oral HPT in the UK, Primodos, contained two hormones – norethisterone acetate (NETA;10mg per tablet) and ethinylestradiol (EE; 0.02mg per tablet). Other oral HPTs marketed in the UK similarly contained high doses of a progestogen and an estrogen.

One Primodos tablet was taken on two consecutive days by women suspected to be pregnant. In women who were not pregnant, a withdrawal bleed would occur a few days later. The total dose of Primodos for diagnosing a pregnancy was therefore 0.04mg EE plus 20mg NETA delivered over 2 days.

NETA, a prodrug of norethisterone (NET), is a progestogen derived from nortestosterone that also has weak oestrogenic and androgenic properties. The action that NET exhibits is therefore complex and will depend on its dose, route of administration, duration of use, the presence or absence of other hormones and the presence or absence of different hormone receptors. One Primodos tablet typically contained about twice the dose of NETA per tablet (but one third lower daily dose) than preparations used today for gynaecological indications and between 2.5 and 10 times more NETA per dose than most combined oral (hormonal) contraceptives (COCs) available at that time and 10 times more than in today's COCs.

EE is a semi-synthetic estrogen with actions similar to those of natural estradiol (E2). EE exerts potent estrogenic effects through its action at the estrogen receptors and has similar or slightly stronger estrogen agonist activity than the naturally occurring estrogens. One Primodos tablet typically contained approximately 5 times lower EE than one combined (hormonal) contraceptive (COC) tablet available at that time and the same amount as one dose of most low dose COCs available in the UK today.

2.3 Overview of the Review by the EWG on HPTs

The report of the EWG (hereafter referred to as EWG report), including copies of the papers and all the evidence considered by the group, was published in November 2017.

The lay summary of the EWG report which sets out the background, scope and overview of the EWG is attached at Annex 1. The full report and its annexes are available on the MHRA website (see Report of the Commission on Human Medicines' Expert Working Group on Hormone Pregnancy Tests – GOV.UK (www.gov.uk)).

For ease of access, extracts of the EWG report that provide relevant background information for the Danielsson paper are included or summarised briefly below and cross-references to specific sections in the main report or annex (hereafter referred to as EWG annex x) are provided as relevant. Further details of the evidence considered by the EWG relevant to the Danielsson hypothesis are provided in section 3 below. A copy of the Danielsson paper is attached at Annex 2.

2.3.1 Summary of data reviewed

Against a background of heightened awareness of the possible teratogenic effect of medicines taken in pregnancy (through experience with thalidomide) a great many studies, letters and reviews have been written on the use of HPTs since they were first introduced to the market. In October 1967, the first observational study to suggest a link between use of HPTs in pregnancy and congenital anomalies in the child exposed *in utero* was published in a letter to the journal Nature (Gal et al, 1967[7]). This study stimulated major research interest in the issue and many further epidemiological studies investigating a possible association between HPTs and a range of congenital anomalies were published thereafter.

The EWG considered evidence from laboratory studies, studies in animals and studies in humans regarding congenital anomalies and miscarriage. The review covered:

- patterns of anomalies reported in babies whose mothers had been given an HPT collected via spontaneous reports to the Yellow Card system or submitted in the call for evidence (including from members of the 'Association for Children Damaged by Hormone Pregnancy Tests')
- patterns of anomalies considered in observational studies using HPTs or hormonal contraceptives or from studies using NET to prevent pregnancy loss.
- evidence for a direct teratogenic effect from studies in animals and humans,
- evidence for an indirect teratogenic effect through disturbance of the pregnancy (vascular disruption),
- evidence for an abortifacient effect of NETA and/ or EE from observational studies using HPTs or hormonal contraceptives or from studies using NET to prevent pregnancy loss.

Many of the studies considered originated from the time HPTs were used to diagnose pregnancy. A critical consideration during the EWG review was that since they were carried out in the 1950s to 1970s, the design, conduct and quality of the studies were largely poorer than would be expected of those conducted today. The EWG therefore set out what would constitute different strengths of evidence to support a causal association between use of HPTs and an adverse outcome of pregnancy for the different types of available data (EWG report, section 3.3) and included a formal quality scoring system of the observational studies. Likewise, key criteria were defined that would need to be met for biological plausibility for a direct pharmacological action (EWG report, chapter 4).

The EWG used a conservative estimate of the likely window for use of Primodos as being from the week of the woman's first missed period to the end of the first trimester; that is, 4 to 12 weeks of pregnancy (2 to 10 developmental weeks).

Chapters 4 and 5 of the EWG report considered the evidence for a causal association with congenital anomalies and chapter 6 considered the evidence for a causal association with miscarriage.

Evidence for a causal association with congenital anomalies (EWG report, chapters 4 & 5)
Regarding *biological plausibility* for a direct pharmacological action, limited evidence was found and only a very small amount of the data came from studies in pregnant women. As a result, a number of assumptions had to be made based on knowledge of how levels of norethisterone and ethinylestradiol change in the blood of (non-pregnant) women given doses broadly similar to those in Primodos.

Based on the available evidence, the EWG concluded that small amounts of norethisterone and ethinylestradiol could have reached the fetus as the result of taking Primodos tablets for two days during the first trimester of pregnancy but that it was unlikely to have had an effect on the developing fetus via a direct pharmacological action. Any action of these hormones would require the expression of functional receptors and would undoubtedly be affected by the relatively high concentrations of the very similar natural maternal estrogen and progesterone in early pregnancy (discussed in EWG Annexes 18 and 19).

Evidence for a direct teratogenic effect
Regarding *evidence for a direct teratogenic effect from animal studies*, a total of 38 published studies and 44 unpublished studies in animals were evaluated to see if there was any evidence for a teratogenic effect with norethisterone or ethinylestradiol (EWG Annex 20). Preliminary findings from researchers at Aberdeen University were also presented to the EWG but were unpublished at the time of the EWG report publication (see section 2.3.2, below).

Consistent findings in mice, rats, guinea pigs and rabbits were shown in these studies. Malformations of the genital tract or genital organs and the abnormal development of male sexual characteristics in a female (known as virilisation) were reported in some rats, mice and non-human primates that were exposed to norethisterone and ethinylestradiol during the period of sexual differentiation late in organogenesis. These effects reflect the known pharmacological action of these compounds and so the review focused on anomalies of non-reproductive tissues, for which there has been scientific uncertainty over evidence for a causal association.

Most studies found very little evidence for an increased risk of malformations in other (non-genital) organs apart from the occurrence of random events, such as cleft palate, absence of one or both eyes (anophthalmia) and a disorder in which the brain is outside of the skull (exencephaly) in a very small proportion of the young. In one study in mice there was evidence that giving a combination of the two hormones throughout the whole period of development at doses approximately 30 times higher than those found in HPTs was associated with an increase in malformations of the chest and mid-body. A similar increase in such malformations was not seen in rats, rabbits or non-human primates. While the effect seen in the mouse was considered to be related to the drug, the effect would therefore seem to be species specific, with the mouse being the sensitive species.

Taking into consideration the quality considerations (EWG report, p25), the EWG concluded that the totality of the available data from studies in rats, rabbits, and non-human primates

did not support a causal association between administering norethisterone and ethinylestradiol at the doses and durations found in Primodos and the development of malformations in non-sexual tissues of the offspring.

Regarding *reports of congenital anomalies in babies of mothers given HPTs*, the number of reports of congenital anomalies in babies of mothers given HPTs was relatively small in comparison to their extensive use. One in six of the babies with anomalies reported as exposed to HPTs had more than one congenital anomaly but no consistent pattern could be identified; for some of these the EWG considered it might now be possible to identify an underlying genetic basis with currently available genetic tests. Some differences were observed between the pattern of congenital anomalies reported with HPTs and the pattern reported to either the EUROCAT congenital anomaly database or MHRA's Yellow Card database with all other medicines, with some anomalies being over-represented and some under-represented, but it was difficult to draw any firm conclusions due to the limitations of this type of reporting.

Overall, the EWG concluded that the available adverse event reporting data had many limitations but did not support a causal association between use of HPTs, including Primodos, during pregnancy and congenital anomalies. Anomalies reported in association with HPTs were largely those that are clearly visible at birth and which occur relatively frequently in the general population.

Regarding *data from observational studies*, the EWG considered 97 studies on use of HPTs by pregnant women. Taking into consideration the quality criteria, in general, the studies were judged to have important limitations in their design and to be of poor quality with respect to at least one (and up to five) of the seven criteria. This made it difficult to draw any robust conclusions: that is, the evidence from many of these studies was insufficiently strong to demonstrate with certainty either that there was a causal association between HPTs and congenital anomalies or conversely that there was no possibility of a causal association. However, after a very careful assessment of each study the following observations were made:

- There was limited evidence for a weak association between the use of HPTs and congenital heart defects, limb reduction defects, and oesophageal atresia, but it was felt this could be due to chance or confounding factors.
- The evidence reviewed did not support an association between the use of HPTs and neural tube defects, orofacial clefts (hare lip or cleft palate), digestive system and abdominal wall defects, skeletal defects (other than limb reduction defects) or overall congenital anomalies but the quality of the evidence is limited.
- From the evidence available it was not possible to draw any conclusions about a possible association between the use of HPTs and urinary system or genital defects, nervous system defects (other than neural tube defects) or VACTERL (Vertebral defects, Anal atresia, Cardiovascular malformations, Tracheoesophageal fistula, Esophageal atresia, Renal anomalies and Limb defects).

Overall, the EWG concluded that while the quality of the available epidemiological evidence was generally very limited, no strong associations were found between the use of HPTs, including Primodos, during pregnancy and any single anomaly, or any pattern of anomalies. The weak associations that were observed could have occurred by chance or confounding.

Taken together, the EWG considered that the complete body of available evidence from pharmacology, non-clinical, epidemiological and adverse event reporting data was very

limited and did not, on balance, support an association between use of HPTs such as Primodos by the mother during early pregnancy and congenital anomalies in the child.

Evidence for an indirect effect on congenital anomalies
Evidence for an *indirect teratogenic effect on through disturbance of the pregnancy* caused by disruption or interruption of the intrauterine blood supply (so-called 'vascular disruption') was considered as another possible mechanism for congenital anomalies (EWG Annex 21). However, no evidence in support of a possible disruptive effect of the components of Primodos on placental blood vessels was identified.

Evidence for a causal association with miscarriage (EWG report chapter 6)
Regarding *evidence for an abortifacient effect* animal studies which reported on loss of the developing embryo (embryo-lethality) were also assessed. Death of the developing embryo with high doses of estrogens has been consistently observed in animal studies and is now considered to be a well-established effect. A similar effect has been observed in studies with norethisterone (or related progestogens). As may be expected, the combination of norethisterone and ethinylestradiol also showed consistent embryo-lethality in different animal species. This effect was dose-dependent and varied according to when and for how long during pregnancy it was given.

In humans, the effect of norethisterone and ethinylestradiol (at doses equivalent to two Primodos tablets taken together) on early human pregnancy was investigated in two small clinical studies in women seeking legal termination of pregnancy in Finland. Though not large enough to detect any small differences in rates of miscarriage between the women who received hormones compared with those who received placebo, no adverse effects were observed on the developing pregnancy in terms of bleeding or a fall in maternal levels of progesterone.

In addition, 21 published studies in women who were given ethinylestradiol or norethisterone or both were evaluated. Most of these studies (a total of 14) were conducted in the 1950s and 1960s to prevent threatened abortion and many were considered to have limitations in their design or analysis. Only two studies investigated the use of Primodos for diagnosing pregnancy. The available epidemiological data were considered to be limited and to provide no evidence for an abortifacient effect of NETA and/or EE when given to pregnant women.

Taken together the EWG considered that while administration of ethinylestradiol and norethisterone, mostly at very high doses or for prolonged periods, can result in embryo-lethal effects in animals, there was no evidence that administration of these hormones at the licensed doses used in Primodos during early pregnancy was associated with an increased risk of miscarriage.

2.3.2 Subsequent reviews of publications

Two academic studies were published shortly after the report of the EWG. These were considered by separate *ad hoc* CHM EWGs and both publications were referred to the EU's Committee for Medicinal Products for Human Use (CHMP) under Article 5(3) of Regulation (EC) No 726/2004 at the request of the MHRA for a scientific opinion.

Ad hoc CHM EWG on Evaluation of new research on the developmental effects of norethisterone acetate and ethinylestradiol in zebrafish embryos
An a*d hoc* EWG considered a study from the Vargesson group (Brown et al, 2018) published in *Scientific Reports*[8] which examined the effects of the components NETA/EE on the

development of zebrafish embryos. The review considered the zebrafish model for evaluating effects of NETA and EE in human pregnancy; the robustness of the study; and whether there were any clinical implications.

The assessment report and minutes of the meeting can be found on the MHRA website (at this link). With regard to clinical implications the zebrafish EWG concluded "Developmental effects occurred at concentrations in the zebrafish embryo that were several orders of magnitude higher than would occur following clinical doses." Consequently, the Group considered that the Brown et al., 2018 study "should be considered with the existing evidence as part of the overall weight of evidence and concluded that the study does not raise any new safety concerns for products in clinical use containing norethisterone acetate and ethinylestradiol."

The EMA CHMP Article 5(3) opinion on the zebrafish evidence can be found at Norethisterone and ethinylestradiol - EMEA/H/A-5(3)/1470 - assessment report (europa.eu). The CHMP review concluded that "overall due to the multiple limitations of the study described in the manuscript (Brown et al., 2018) the results of this study do not add to the current knowledge regarding adverse events in early pregnancy in human. The CHMP concluded that there are no new clinical implications based on the results of the presented zebrafish study."

Ad hoc CHM EWG on Systematic Review and Meta-Analysis of studies on Oral Hormone Pregnancy Tests

The second, a study by Heneghan et al[9] published in the online journal *F1000 Research,* concluded that use of oral HPTs in pregnancy is associated with increased risk of congenital anomalies. This was a systematic review and meta-analysis of observational case-control and cohort studies that included data from pregnant women that were exposed to oral HPTs within the estimated first three months of pregnancy and compared with a relevant control group.

Assessor's comment: The Henegan meta-analysis included a slightly different dataset to the EWG analysis because the inclusion and exclusion criteria differed slightly. Nine studies included in the analysis of the EWG were not included in the analysis by Heneghan et al, while two studies included in Heneghan et al were not included in the EWG's analysis. Overall, the data in both were substantively the same.

The assessment report and minutes of the meeting can be found on the MHRA website (at this link). The CHM systematic review EWG concluded the Henegan "study could not be considered robust due to limitations in the design, reporting and analysis of the included studies and there would be little value in re-analysing the data".

The EMA CHMP opinion can be found at Norethisterone and ethinylestradiol - metanalysis - EMEA/H/A-5(3)/1477 - assessment report (europa.eu). The CHMP concluded "the quality of most studies used is questioned and, as a result, the conclusions of the meta-analysis cannot be considered reliable. Due to the multiple limitations of the meta-analysis study, the results described in this manuscript cannot be used to further expand clinical knowledge. The results of this meta-analysis, thus, have no clinical implications. As a consequence, the conclusion that current clinical data available do not support a signal of teratogenicity of a combination of norethisterone/ ethinylestradiol remains valid. The CHMP therefore did not recommend any further regulatory actions based on the above data."

3 NEW INFORMATION - DANIELSSON ET AL (2023) REVIEW

The abstract of the Danielsson review states its aim is "to review and compare the pattern of malformations produced by transient embryonic hypoxia of various origins in animal studies with malformations associated with transient embryonic hypoxia in human pregnancy due to a failed abortion process."

This is expanded on in the text (p2):
"One aim of this review is to present evidence that ROS generation related to transient embryonic hypoxia, and particularly in conditions of massive ROS generation when oxygen returns to severely oxygen-deprived embryonic tissues, can induce a similar spectrum of stage specific teratogenicity across species, including humans."
"Another major aim is to compare the pattern of malformations produced by transient embryonic hypoxia of various origins in animal studies (by low oxygen tension, by clamping of uterine vessels or by inducing severe irregular cardiac rhythm in the embryo) with the pattern of malformations associated with transient embryonic hypoxia in human pregnancy."

In relation to HPTs the Authors propose:

- the results show transient hypoxia and compounds with potential to cause failed abortion in humans, are associated with a similar spectrum of teratogenic outcomes
    - teratogenic outcomes include limb reduction, cardiovascular and central nervous system defects

- HPTs like Primodos as examples of drugs that can induce failed abortion

- a common teratogenic mode of action for HPTs and drugs causing failed abortions (such as misoprostol) is likely to be secondary to uterine contractions and compression of uterinoplacental/ embryonic vessels during organogenesis.

3.1 New data highlighted

The article provides a comprehensive review of the harmful effects of reactive oxygen species on embryonic tissues. The authors highlight "A wide range of other malformations can be produced by temporary hypoxia in animals; the type of embryofetal manifestions induced is dependent on the degree and severity of the hypoxia, but also on the stage of embryofetal development when the hypoxia event occurs".

Points to consider:
1. Hypoxia followed by re-oxygenation is well understood by those working in the field of pathology, including teratology, as a potential mechanism of damage following interruption of an oxygenated blood supply such as might occur following vascular disruption or uterine trauma. It is not a unique concept to teratology and is established in many other fields of pathology.
- Hypoxia appears to be the latest mechanism theorised to explain vascular disruption type birth defects but this was not the only proposed mechanism and it is not clear how much early observations were due to gross mechanical damage or other explanations

Assessor Comment:
Harmful effects from oxidative stress are a well-recognised phenomenon and has been proposed as a mechanism for adverse effects in pregnancy, particularly in connection with

placenta-related conditions such as pre-eclampsia and miscarriage. As such, the *potential* for oxidative stress to adversely affect a pregnancy is accepted. This was noted but not considered in detail by the EWG.

The EWG review found no evidence that the components of Primodos induced vascular disruption. This paper focuses on whether the Danielsson review presents any new or direct evidence that HPTs induce oxidative stress or the precursor steps (uterine contractions and/or vascular disruption).

3.2 Miscarriage or failed abortion

Danielsson et al, propose there is evidence that HPTs have the potential to initiate a failed abortion process, resulting in uterine contractions, bleedings and attempts to expel the uterine endometrium (with the conceptus) in a similar way to when menstruation occurs in non-pregnant women. They suggest plasma concentrations of progesterone in some pregnant women in early pregnancy are lower than in non-pregnant women and thus HPTs could initiate an abortion process in some pregnant women with lower progesterone levels than in non-pregnant women.

In support of the hypothesis that HPTs initiate uterine contractions and a failed abortion process, the authors cite one human clinical study with HPT (ethisterone + ethinyl estradiol) conducted by Rawlings (their Ref 12, pages 2, 8 and 9).

P2: "Both misoprostol, as reviewed by Auffret et al. [11], and HPTs [12] have been reported to initiate uterine contractions and a failed abortion process in organogenesis with signs the same as those in early threatened abortion (contractions and bleeding) in some pregnant women"

P8: "Several investigators have proposed these abortifacient drugs act by causing uterine contractions leading to compression of uterine and/or embryofetal vessels and embryonic hypoxia during the failed abortion process to underlie the teratogenicity as reviewed by Auffret et al. [11]. This is highly interesting in view of evidence that Hormone Pregnancy Tests (HPTs) have been reported to cause similar signs such as in early threatened abortion in some pregnancies (uterine contractions and vaginal bleeding), but the pregnancy continues [12]."

P9: "There is evidence indicating that HPTs have the potential to initiate a failed abortion process, resulting in uterine contractions, bleedings and attempts to expel the uterine endometrium (with the conceptus) in a similar way as when menstruation occurs in non-pregnant women [12]. The plasma concentrations of progesterone in some pregnant women in early pregnancy (range 10–44 ng/ml) are lower than in non-pregnant women (range 2–25 ng/ml) [122]. These results imply that HPTs have the potential, and could be expected, to initiate an abortion process in some pregnant women with lower progesterone levels than in non-pregnant women."

The authors highlight "Five out of 66 women with 5–8 weeks amenorrhea using a HPT for two days showed neither menstruation bleed (= "not-pregnant") nor absence of bleeding (="pregnant"); rather they showed 'spotting' and signs similar to in(sic) early threatened abortion. Three of these women were subsequently proven to be pregnant; this means that three out of a total of 35 pregnant women using HPTs showed clinical signs such as in early threatened /failed abortion, but the pregnancy continued". The authors conclude "This failed

abortion mechanism is suggested as the same as the mechanism proposed to cause teratogenicity for the established human teratogen misoprostol."

The study by Rawlings (1960[10]; annex 3) was a small clinical study (n=66 women with 5–8 weeks amenorrhea) comparing the reliability of the toad test for pregnancy with the HPT 'orasecron' (5 tablets of ethisterone + ethinylestradiol (one every 4 hours) on 2 consecutive days then reviewed 1 week later). All women took the HPT and an unspecified number of participants had a history of previous abortion (miscarriage).

- 36 women had no bleeding and were later confirmed to be pregnant;
- 5 women reported spotting only (slight brown staining) of whom 3 had positive toad tests and went on to pregnancy and 2 had negative toad tests and were later confirmed as not pregnant.
- 2 women were showing signs of threatened abortion at the time of testing (blood loss per vaginam). In one, these signs subsided within two days, although the patient gave a history of previous abortions and the pregnancy proceeded to term. The other patient had an abortion during the trial, and histological examination showed an early precursor of hydatidiform mole.

Rawlings concluded "It seems that "Orasecron" has no harmful effect on pregnancy (many patients treated had a history of previous abortion), and because of its ethisterone content may even be beneficial in a case of threatened abortion."

Points to consider.

1. Rawlings (1960) provides information on bleeding patterns and pregnancy rates but no other information on pregnancy outcomes or uterine contractions is reported. This paper was not included in the EWG HPT review, presumably because it did not reach the criteria for inclusion in the review of epidemiology papers.

- The study did not apparently consider the cases of spotting as threatened abortion since the publication distinguishes between these and 2 cases of threatened abortion.

2. Bleeding and spotting are estimated to be common in early pregnancy, especially around time of implantation, with subchorionic haematomas, with post-coital bleeding, with infections, and with ectopic or molar pregnancies.

- Hasan et al (2010)[11] estimated around 25% of 4539 participants reported bleeding and about 8% reporting heavy bleeding in early pregnancy. Most episodes lasted less than 3 days, and most occurred between gestational weeks 5–8. Twelve percent of women with bleeding and 13% of those without experienced miscarriage. Maternal characteristics associated with bleeding included fibroids and prior miscarriage.

3. The EWG considered the available clinical evidence for miscarriage with HPTs (EWG Chapter 6 and Annex 30)

- A total of 21 studies of HPTs were considered for miscarriage, including 2 studies using EE with NETA, 2 with EE and other progestogens, 4 EE without progestogens and 11 studies using NETA without EE. Some of the studies were case series, with no comparator groups and most of the published papers identified concerned the use of EE and/or NETA for threatened abortion.
- None of the 4 HPT studies reported an abortifacient effect with all rates within the 5-20% standard range.
- The EWG concluded that available epidemiological data were limited and provide no evidence for an abortifacient effect of NETA and/or EE when given to pregnant women.

- Maternal bleeding is considered in EWG Annex 29 (mainly as a confounding factor in studies of threatened abortion) and EWG Annex 21 (including a small clinical study (Pulkkinen et al, 1984) which looked directly for placental changes following intake of Primodos).

Non-clinical evidence for miscarriage/abortion

Danielsson et al highlight "initiation of an abortive process has also been reported in several studies of human relevant primate animal studies after administration of HPTs. Repeated dosing resulted in a low number of fetuses examined at term (4–10 per group) due to high incidences of abortions e.g. 52–60% in Cynomolgus monkeys and 20–67% in Rhesus monkeys [123–126]."

Points to consider.

1. The EWG considered the rates of embryo-fetal loss through either uterine resorption in rodents and rabbits or abortion in primates following dosing with NETA/EE combinations (Chapter 6 and Annex 20). The publications reviewed include the cited references 123-125 (Ref 126 is the EWG report).
- There was evidence of differences in species sensitivity to Primodos. Based on delivered dose per day (not exposure) to mice, and rats the pregnancy terminating effects of combinations of NETA/EE were above the Primodos dose when the dose equivalent was estimated conservatively based on body surface area and throughout organogenesis (see EWG report, Table 18).
- Rabbits were more sensitive, and some embryo loss was seen at similar estimated doses and dose durations to those used for Primodos.
- Data from non-human primates, the most physiologically relevant animal species, showed significantly increased embryo lethality over controls at 100-1,000 x the Primodos dose and a possible small increase in pregnancy loss at around the equivalent Primodos dose when given daily for 30 days during early pregnancy.

The animal studies were considered by the EWG to have several limitations:
- No animal study replicated the dosing regimen used for Primodos in early pregnancy and most animal studies dosed throughout organogenesis.
- Plasma exposure of the administered hormones and their respective active metabolites were not measured and so direct comparison with human exposures to Primodos was not possible.
- Use of rodents and rabbits to study reproductive effects of sex steroids is limited due to differences in hormonal and reproductive physiology, early embryogenesis and placental structure compared to humans.

The EWG in reaching its conclusions gave much greater weight to the clinical and epidemiological evidence. Overall, the EWG concluded that while administration of ethinylestradiol and norethisterone, mostly at very high doses or for prolonged periods, can result in embryo-lethal effects in animals, there was no evidence that administration of these hormones at the licensed doses used in Primodos during early pregnancy were associated with an increased risk of miscarriage.

3.3 Patterns of teratogenicity

Danielsson et al, provide a summary of epidemiology studies (their Table 2) that investigated the teratogenic potential of HPTs in the 1970s and 80s and reference the further reviews by the HPT EWG and the Hennigan et al 2019 paper that was considered by the CHM ad hoc EWG and the EU CHMP.

The authors state "conducted epidemiological studies show an increase of malformations in the nervous system (neural tube defects, including spina bifida and encephalocele, and anencephalus), cardiovascular system and limbs (particular limb reduction defects of various severity), and also an increase in orofacial clefts, atresia in the gastrointestinal system (oesophageal atresia), renal defects (unilateral renal agenis, bladder extrophy) and a syndrome with combined anomalies such as the VACTRL association. VACTRL is defined when at least three of the following anomalies are present: Vertebral defects, Anal atresia, Cardiovascular anomalies, Tracheoesophageal fistula, Esophageal atresia, Renal anomalies, and Limb defects."

The authors also highlight the Henegan et al meta-analysis (reviewed by the 2019 *ad hoc* CHM EWG and the EU CHMP) which they highlight "showed a statistically significant increase in anomalies at these organ system levels (nervous-, cardiovascular-, musculoskeletal, renal- and gastrointestinal system) as well as combined anomalies, such as VACTRL."

Points to consider:

1. Table 2 tabulates the anomalies reported in human studies. The table includes both individual epidemiological studies and reviews of relevant studies:
   - studies which found increased rates of specific congenital anomalies or syndromes are highlighted but information on (numbers of) studies which investigated these is not included
   - study type (case-control, cohort, narrative review or meta-analysis) and duplicate or overlapping studies are not identified
   - the table includes the Henegan meta-analysis
2. The EWG considered the available clinical evidence for congenital anomalies with HPTs (EWG Chapter 5, Annex 27 and Annex 29). This included all the studies cited in Table 2 (including Janerich 1977[12] [mis-cited as Gal 1977, reference 145]) except ref 139 which is the EWG report and 121 which is the Henegan meta-analysis which was reviewed by a standalone ad hoc EWG.
   - There was limited evidence for a weak association between the use of HPTs and congenital heart defects, limb reduction defects, and oesophageal atresia, but it was concluded this could be due to chance or confounding
   - The evidence reviewed did not support an association between the use of HPTs and neural tube defects, orofacial clefts, digestive system and abdominal wall defects, skeletal defects (other than limb reduction defects) or overall congenital anomalies in the fetus but the quality of the evidence is limited
   - From the evidence available it was not possible to draw any conclusions about a possible association between the use of HPTs and urinary system or genital defects, nervous system defects (excluding neural tube defects), or VACTERL
   - The EWG concluded 'While the quality of the available epidemiological evidence was generally very limited, no strong associations were found between the use of HPTs, including Primodos, during pregnancy and any single anomaly, or any pattern of anomalies. The weak associations that were observed could have occurred by chance or confounding.'

The Authors go on to state that in considering teratogenicity "it is important to also study subgroups of malformations and not only an increase at the organ system level." They highlight the EWG analysis which identified a small dataset (n=173) derived from several sources of HPT-exposed patients cases with major congenital anomalies that were classified according to the EUROCAT method as discussed in Annex 24 of the EWG report.

Danielsson takes data of anomalies reported for HPTs from the EWG report to produce a descriptive list of 10 anomalies showing an increase compared to EUROCAT data (main text page 10 column 2) and point to a number of rare combinations of malformations which they suggest were not discussed by the EWG including Moebius syndrome, Klippel- Feil syndrome (mainly characterised by vertebrae defects), and combined nervous system and limb defects, unilateral absence of the kidney and VACTRL in children with multiple malformations.

Points to consider:

1. Individual or case series of reports of congenital anomalies are usually considered as *suspected* adverse drug reactions (ADRs) and are not usually directly compared to data from epidemiological studies.

- Adverse drug reactions for marketed medicines are reported when the reporter suspects they may be associated with the exposure. For exposures in pregnancy, this includes suspected adverse drug reactions in the mother, developing fetus or child. However a report does not necessarily mean that the medicine was responsible for that event or reaction. Other factors may have caused or contributed including the person's genetic makeup, maternal nutrition, their underlying health and pre-existing or new onset illnesses and/or other concomitant medications.
- Congenital anomalies were diagnosed in 222 per 10,000 births in England in 2020[13].
- Suspected ADRs are referred to as being reported *in association with* an exposure but such reports alone cannot usually establish if there is *a causal association* ie differences in risk compared to rates in those who were not exposed. Rather such reports usually prompt further epidemiological studies.

2. Regarding the references to the dataset of HPT-exposed cases considered by the EWG

- the origin of some of the data cited by Danielsson et al, on congenital anomalies is unclear as this is referenced to EWG Annex 24 but there are some discrepancies in comparative rates of ADR reports cited (for HPT exposure compared to EUROCAT database) compared with EWG Annex 24 (the cited reference) and with the EWG report Table 14 (EWG report page 55); no details of the author's method of (re-) analysis are provided to explain the discrepancies
- the text suggests that some syndromes were observed but excluded from the EWG analysis – some were excluded from the EWG analysis after being recoded and/or classed as of genetic origin according to EUROCAT criteria (EWG Annex 24)

3. Regarding the EWG review of the HPT dataset (EWG Annexes 22, 23 and 24):

- Many of these reports were limited by lack of medical confirmation or insufficient case details, and the majority were reported retrospectively.
- Comparison with the EUROCAT and BINOCAR databases was undertaken to see if any anomalies had been reported more frequently in association with HPTs than might be expected in the general population.
- Comparison of the pattern of congenital anomaly reports in the offspring of women who were given HPTs, with the pattern of anomalies reported to the EUROCAT (international)

and BINOCAR (national) databases showed a higher proportion (≥2 fold) in about 30% of anomalies. In particular a higher proportion of cases specifically describe 'limb reduction defects' in the HPT-exposed dataset (7.3% v s 2%) vs the EUROCAT database.
However, the limitations of the data did not allow any firm conclusions to be drawn and the EWG concluded that these differences could have occurred as the result of chance.

Comparison of subtypes of human anomalies associated with use of HPTs and misoprostol

Danielsson et al propose the pattern of subtypes of HPTs in the EUROCAT analysis is very similar to the pattern of subtypes of malformations reported for misoprostol (their section 5.2.3, Table 3). Furthermore, they suggest most of the malformations are known to be possible to be induced by exposing the embryo to transient periods of interrupted oxygen supply to the embryo followed reperfusion/reoxygenation strongly supporting a common mechanism related to embryonic hypoxia/ROS for HPTs and misoprostol.

The authors state: "Misoprostol, which causes abortions mainly by contracting the pregnant uterus in humans and monkeys [119]"

Points to consider:

1. Vascular disruption and oxidative stress can occur at any time in pregnancy.
- Any effects on the developing fetus will depend on the organs developing at that time and the site of vascular disruption
- The degree of similarity between any resulting congenital anomalies would likely depend on similarity of exposure times, durations and severity of the 'insults'.

2. The ability of misoprostol to induce uterine contractions is recognised in the authorised indication for induction of labour and as a component of medical abortion.

- Misoprostol is a prostaglandin analogue, with apparently selective activity for prostaglandin E receptors.

3. Table 3 lists anomalies reported in the literature *in association with* misoprostol or the HPT dataset
- the table includes both case series and comparative studies without further identification for individual anomalies
- duplicate or overlapping studies and study type (case series, case-control, cohort, narrative review or meta-analysis) are not identified
- the entries for HPTs appear to include literature reports and not solely those from the HPT dataset as the table indicates.
- Reference 119 (Micks et al 2012[14]) describes a protocol for medical termination of pregnancy in cynomolgus monkeys. Misoprostol was administered after mifepristone or methotrexate and the study did not explore mechanism of action.

4. The EWG considered the actions of misoprostol as an example of substances considered to cause vascular disruption (EWG Annex 21, section 3.2 & annex III)
- several of the cited studies are case series lacking a comparator group;
- most of the studies reported birth defects observed in association with abortion failure following its off-label use for self-attempted abortion in Brazil where abortion is illegal

- many of the studies lacked reliable information on dose or timing of exposure
- some of the studies highlighted that more than one method (e.g. other available drugs or physical methods) may have been used to induce abortion, either in addition to misoprostol use, or later, when misoprostol was perceived to have failed to induce an abortion.
- In some studies which reported uterine cramps and /or bleeding following misoprostol use for abortion, uterine cramps with or without bleeding occurred in around 30% or less of subjects.
- Various mechanisms for misoprostol inducing congenital anomalies have been proposed but no studies that specifically investigate the mechanism of congenital anomalies were identified for the EWG review.

Review of results in animal teratology studies by HPTs (Danielsson 5.2.4 page 10-11)

Danielsson et al, highlight that teratology studies in rodents and rabbits exposed to HPTs did not show any clear evidence of an increase in malformations but clear evidence of an increase in resorptions.

The authors consider conventionally designed teratology studies in rodents and rabbits which dose throughout organogenesis are not relevant for detection of human teratogenicity mediated via a failed abortion process. This is because in rodents during organogenesis a dead conceptus undergoes gradual degradation followed by maternal reabsorption rather than being expelled via uterine contractions (abortion), similarly rabbits resorb dead embryos during early organogenesis although can abort dead embryos later in organogenesis.

The authors propose "the relevant mechanism for induction of teratogenicity in humans, mediated by expulsion of an embryo via uterine contractions (abortion) is nonexistent in rodents during whole organogenesis and in rabbits in early organogenesis. Instead the conceptus undergoes gradual degradation followed by maternal resorption in the uterus."

The authors state that the initiation of an abortive process has also been reported in several studies in human-relevant primate animal models after administration of HPTs. They highlight a cynomolgus monkey study with an HPT where "one out four (sic) examined fetuses was malformed in the low dose group in a cynomolgus study [124], showing rib and vertebral malformations of a similar type as associated with failed abortion after use of HPTs in humans". The authors go on to note "However the low number of surviving fetuses is insufficient to evaluate a teratogenic potential." and "species differences can explain why a number of teratology studies in rodents and rabbits exposed to HPTs did not show any clear evidence of an increase in malformations but a clear evidence of an increase in resorptions (=embryonic death) at human relevant exposures of HPTs".

According to Danielsson whilst embryofetal effects can be observed in rats via mechanisms inducing transient hypoxia (their Table 1), teratogenic effects will not be observed in rats or rabbits in early organogenesis if the hypoxic event is induced via uterine contractions. Thus, by contrast, when dead fetuses are expelled via uterine contractions (abortion), such as with primates or in rabbits in late organogenesis, both embyrofetal loss and teratogenicity should be observed.

Points to consider:

1. Embryofetal resorptions occur in rodents and rabbits during early organogenesis whereas abortions occur in primates

- abortion is defined by expulsion of a dead fetus rather than the occurrence of uterine contractions
- uterine contractions occur in rodents at parturition, it is presumed that uterine contraction does not occur when embryofetal resorption occurs but direct evidence for this is lacking

2. In conventional developmental toxicity studies, the uterus is not examined until the end of gestation generally making it difficult to determine from evaluation of the products of conception whether or not intrauterine deaths were due to malformations

- For teratogens that induce significant embryofetal loss in conventional teratology studies, severely malformed fetuses may die and not be detected when the uterine contents are examined at the end of the study. However, increased fetal malformations can generally be observed at lower doses (ie below those where significant embryofetal loss is observed) and relatively severely malformed fetuses can still survive to late gestation and be detected.

3. Table 1 of the Danielsson paper provides an overview of major malformations produced by transient hypoxia of varying origin in rats.

- Procedures in rodents which induce vascular disruption via uterine clamping led to a range of malformed fetuses which survived to late gestation or term (Webster et al, 1987[15]). Similarly for example with class III antiarrhythmic drugs, lower doses than those causing embryo lethality induced gestation day specific teratogenic effects (Wellfelt et al, 1999[16]) (both cited by the authors [55] and [58] respectively).
- Ref 124 highlighted by Danielsson et al refers to Hendrickx et al, 1987(b)[17], a non-human primate teratology study. This reference appears to be miscited and likely intended as reference [123] Hendrickx et al 1987 (a)[18].
- In three studies, NETA and EE (Hendrickx et al 1987a) were given orally at doses ranging from 1 to 1000 times the Primodos dose (on a mg/kg/day basis) to rhesus monkeys, cynomolgus monkeys or baboons over a 30-day period, from gestation day 20 to 50 (the period of organogenesis). The number of pregnancies evaluated in each test group varied from 9-21 and whilst there was significant embryofetal loss in some dosed groups the studies were sufficiently sensitive to detect an increase in genital malformations at the high doses but only one case of non-genital anomalies was detected (defects of the ribs and the vertebrae) in cynomolgus monkey low dose group (100x Primodos dose) with 10 surviving fetuses. The EWG acknowledged these studies may not have sufficient power to observe an increase in rare events.
- The authors appear to be suggesting that hypoxia induced teratogenicity is not seen in rodents and rabbits studies with HPTs, because the absence of uterine contractions during embryofetal resorption means no hypoxic events occur during “failed” abortions. In this hypothesis, the occurrence of uterine contractions is necessary for malformations to occur. This contrasts with the conventional view that malformation effects are potentially masked by embryofetal loss.

4. The HPT EWG review considered the overall review of animal teratogenicity studies by the EWG (EWG Annex 20):

- The EWG also considered the results of industry sponsored teratology studies conducted in rats and rabbits designed to test the hypothesis that after treatment of a combination of NETA and EE spanning different periods of organogenesis, teratogenic effects might occur, which otherwise might have been masked by a more markedly embryo lethal effect observed after treatment throughout organogenesis.
  - These studies included dosing in rabbits late in organogenesis where the potential for

uterine contractions and abortive processes exist.
- Abortions were observed in some studies but were considered in line with normal background rates.
- These studies did not reveal an increase in malformations relative to controls across different exposure times during organogenesis in rats or rabbits (EWG report Chapter 6, Annex 20).

- The HPT EWG with regards to animal teratology studies concluded that the evidence from studies in rats, rabbits, and non-human primates did not support a causal association between administering norethisterone and ethinylestradiol at the doses and durations found in Primodos and the development of malformations in non-reproductive tissues of the offspring.

3.4 Vascular disruption

Danielsson et al, (their section 5) cite misoprostol and chorionic villus sampling (CVS) as examples of 'insults' that result in vascular disruption in humans and highlight the similarities in malformations reported in association with these insults: "The pattern of the prominent malformations associated with CVS in the literature [156–158] is very similar to the pattern of the most prominent malformations associated with HPTs and misoprostol". The authors also highlight that uterine manipulation or trauma in human pregnancy can result in vascular disruption for the developing fetus.

Points to consider:

1. Vascular disruption and oxidative stress can occur at any time in pregnancy.

- Any effects on the developing fetus will depend on the organs developing at that time
- Vascular disruption effects can be one-sided depending on the vessels affected
- The degree of similarity between any resulting congenital anomalies would likely depend on similarity of exposure times, durations and severity of the 'insults' and the antioxidant capacity of the surrounding tissue.
- 'Vascular disruption' has been cited by some authors as a diagnosis of exclusion (i.e., when no alternative cause can be identified).

2. Potential for HPTs to disrupt an ongoing pregnancy were considered in EWG report section 5.1.4 and Annex 21, section 4.

- The main examples provided by Danielsson et al, in relation to embryonic hypoxia, namely, uterine trauma, Chorio Villus Sampling (CVS), and misoprostol were specifically examined by the HPT EWG along with the evidence for initiation of an abortion process and risk of vascular disruption as potential causes of birth defects in association with HPT use. (Annex 21,, section 3).
- Oxidative stress was mentioned briefly in Annex 21 (annex I) but not considered in detail since it this was accepted as a possible mechanism for placental damage which would occur as a downstream mechanism if vascular disruption occurred.

3. Regarding the potential for vascular disruption The EWG review specifically considered effects of NETA:

- analogous to progesterone withdrawal and progesterone antagonism
- to cause vaginal bleeding and disruption of endometrial lining / uteroplacental structure
- to induce of uterine tone and/or contractions
- to cause acute reductions in maternal blood flow through the uterine artery vasoconstriction / placenta
- to cause induction of a blood clot within the embryofetal circulation

- No studies of uterine tone or contractions flow during exposure to EE and / or NET were identified
- No direct evidence was identified by the EWG review that NET actually induces signs of an abortifacient effect or vascular disruption which could lead to effects at doses relevant to HPT use.

4 DISCUSSION

The authors hypothesize that HPTs (i) cause 'failed abortion' (ii) that the uterine contraction arising from the failed abortion causes hypoxia-induced teratogenicity in the fetus. The authors base their hypothesis on analogy to teratogenicity reported after CVS and failed abortions with misoprostol.
The authors appear to be suggesting that hypoxia induced teratogenicity is not seen in rodents and rabbits studies with HPTs, because the absence of uterine contractions during embryofetal resorption means no hypoxic events occur during "failed" abortions. In this hypothesis, the occurrence of uterine contractions is necessary for malformations to occur. This contrasts with the conventional view that malformation effects are potentially masked by embryofetal loss in such species. However, malformations would be seen in species such as humans which experience uterine contractions as part of the abortion process.

To consider whether the review has any implications for the previous conclusions of the HPT EWG several aspects are considered here.

Evidence for HPTs to cause abortion
Danielsson et al refer to teratogenicity arising from *compounds with potential to cause failed abortion in humans*. Whilst products which cause abortion (abortifacients) may also lead to failed abortion (which would be equivalent to 'treatment failure'), it is unclear to what extent non-abortifacients can be considered to 'cause' failed abortion, without causing abortions too.

The HPT EWG considered evidence for NETA and/or EE to cause abortion, including from studies where NETA was used to avert a threatened abortion, and concluded that available epidemiological data were limited and provide no evidence for an abortifacient effect of NETA and/or EE when given to pregnant women.

The authors cite a single study (by Rawlings (1960), using EE and ethisterone) to support the claim that HPTs cause failed abortion. The study included 66 women of whom an unknown number had a history of previous miscarriage. Danielsson et al consider that cases of spotting were signs of threatened abortion. Notably however the original publication distinguishes between these and 2 cases of threatened abortion, suggesting the study did not apparently consider the cases of spotting as threatened abortion according to definitions at the time and one case of threatened abortion went to term.

Nevertheless, the study was not designed to look at miscarriage or failed abortion and there was no untreated comparison group to assess (albeit with very low power) the rates of 'failed abortion' for women who took the HPT compared to those who did not. Bleeding and spotting in early pregnancy is common and this small study identified by the authors does not add relevant data to the evidence on risk of abortion with HPTs considered by the EWG.

Evidence for HPTs to cause uterine contractions

No studies of uterine tone or contractions following exposure to EE and / or NET were identified by the EWG. Danielsson et al cite the same small study (by Rawlings, 1960) to support the claim that HPTs induce uterine contractions, however the publication for that study does not include any information on cramping or uterine contractions. This publication therefore adds no new information to that considered by the EWG regarding uterine contractions.

The occurrence of uterine contractions is a central tenet of the Danielsson et al hypothesis that HPTs cause congenital anomalies via hypoxia-induced teratogenicity and as an explanation of why teratogenic effects are not observed in animal species that resorb rather than abort a fetus. The absence of data to support that HPTs cause uterine contractions weakens their hypothesis that HPTs cause hypoxia-induced teratogenicity.

Analogy to misoprostol and CVS
The article by Danielsson et al relies heavily on similarities of congenital anomalies reported in association with misoprostol and HPTs. For both, the authors have cited reports of congenital anomalies without clearly distinguishing which of these were reported in case series, or from comparative studies which have suggested an increased risk. The listed anomalies also occur in the general population (i.e., not exposed to either substance), although rates may vary in different regions. Thus without this distinction, equal weight is given to reports which may be due to alternative explanations or occur by chance rather than focusing on those which have been observed at higher rates following exposure to misoprostol or HPTs than might be expected from the general population. This in turn increases the list of 'similar' anomalies.

The EWG considered the studies reporting congenital anomalies following use of misoprostol as an example of substances considered to cause vascular disruption. The EWG review noted a number of limitations in the data on misoprostol and congenital anomalies, including that no studies were identified that specifically investigated the mechanism of how misoprostol induces congenital anomalies.

An unstated assumption of the Danielsson paper is that uterine contractions are a cause of fetal loss rather than a reaction to fetal demise. It is clear that misoprostol induces uterine contractions (a property which underlies its licensed use for induction of labour and as a component of medical abortion). However misoprostol is a prostaglandin analogue and activates E1 receptors. As such, it displays different pharmacological properties to progestogens including NETA. It is unclear whether these different properties contribute to misoprostol's effects to induce abortion and/or congenital defects.

Thus, comparison of raw reports and a different pharmacological action of misoprostol versus HPTs does not provide evidence for concluding that HPTs are teratogenic in the absence of direct evidence that HPTs cause vascular disruption.

Absence of teratogenic effects in rodents and rabbits:
Danielsson et al do not appear to dispute EWG's conclusions that there is an overall absence of teratogenic effects with HPTs in studies with rodents and rabbits. The difference is in the proposed reason for this. The author's suggestion that due to the absence of uterine contractions, hypoxia-related teratogenic effects are not observed in animal species that resorb rather than abort a fetus is a new hypothesis. Limited examples of pharmaceutical interventions (misoprostol, mifepristone and HPTs) are provided in support of this hypothesis, based on reported congenital anomalies. No direct data are cited in support of

this hypothesis, rather it presupposes that the examples given (misoprostol and HPTs) do cause hypoxia-related teratogenic effects in humans that are missed in animal studies. As such the hypothesis is currently unsubstantiated. Additionally, although not explicitly stated in regulatory guidance, regulators are aware of the potential of abortifacients to mask teratogenic effect. Any substance that increases the risk of abortion would be labelled for avoiding unintentional use in pregnancy.

5 CONCLUSIONS AND RECOMMENDATIONS

Danielsson et al propose a new hypothesis that hypoxia-related teratogenic effects are not observed in animal species that resorb rather than abort a fetus due to the absence of uterine contractions. This contrasts with the conventional view that resorption of a fetus after fetal demise may mask congenital anomalies. No direct data are cited in support of this hypothesis, rather it presupposes that the examples given (misoprostol and HPTs) do cause hypoxia-related teratogenic effects in humans that are missed in animal studies.

Notwithstanding a hypothetical mechanism, in reaching their conclusions on HPTs, the EWG placed more weight on the clinical and epidemiological data than on the non-clinical studies. The EWG concluded that while the quality of the available epidemiological evidence was generally very limited, no strong associations were found between the use of HPTs, including Primodos, during pregnancy and any single anomaly, or any pattern of anomalies or on the risk of pregnancy loss. The weak associations that were observed could have occurred by chance or confounding. The new evidence regarding abortion following exposure to HPTs included in the article by Danielsson et al does not add relevant data to the evidence considered by the EWG.

The EWG specifically examined the possibility of HPTs causing vascular disruption. Very little relevant data to the potential effects of NET and EE on the vasculature in pregnancy were identified and the EWG concluded “No evidence that norethisterone and/or ethinylestradiol could disturb a pregnancy through vascular disruption was identified”. Few additional references relevant to vascular disruption with HPTs are cited and several of these do not support the points claimed by the authors. Of note, no new or direct evidence has been identified by the authors to substantiate that HPTs induce oxidative stress or the precursor steps of uterine contractions and/or vascular disruption.

The data presented in the review by Danielsson et al do not add relevant data to the evidence reviewed by the HPT EWG, consequently no further action is proposed at this time.

6 ADVICE SOUGHT

Does the CHM consider that the publication by Danielsson et al presents any new evidence that EE and/or NET could disrupt a pregnancy through vascular disruption?

If so, does the CHM consider that further review by an ad hoc EWG is warranted?

7 LIST OF ANNEXES

1 Lay summary of HPT EWG report
2 Danielsson et al (2023) paper
3 Rawlings (1960) paper

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