TOX/2022/50

Committee on the Toxicity of Chemicals in Food, Consumer Products and the Environment

The Potential Health Effects of Raspberry Leaf in the Maternal Diet

Introduction

1. The Scientific Advisory Committee on Nutrition (SACN) last considered the maternal diet and nutrition in relation to offspring health in its reports on 'The influence of maternal, fetal and child nutrition on the development of chronic disease in later life' (SACN, 2011) and on 'Feeding in the first year of life' (SACN, 2018). In the latter report, the impact of breastfeeding on maternal health was also considered. In 2019, SACN agreed to conduct a risk assessment on nutrition and maternal health, focusing on maternal outcomes during pregnancy, childbirth and up to 24 months after delivery.

2. SACN agreed that, where appropriate, other expert committees would be consulted and asked to complete relevant risk assessments. A provisional list of chemicals was proposed by SACN Members. However, this was subject to change following discussion by the COT. A scoping paper was presented to the Committee (TOX/2020/45) to define the scope of the work from a toxicological safety perspective and also requesting their input on the selection of candidate chemicals or chemical classes that could be added or removed.

3. As part of this work, the Committee decided it would be useful to consider the use of dietary supplements during pregnancy. A discussion paper (TOX/2020/51) was presented, reviewing the commonly used dietary

supplements used during pregnancy. These were supplements that are not officially recommended by relevant authorities but are promoted by anecdotal evidence and unofficial sources as having various purported benefits.

4. The review was confined to herbal dietary supplements which would be regulated under food law, as opposed to traditional herbal medicines, which are overseen by the Medicines and Healthcare Products Regulatory Agency (MHRA). Following this review, the COT suggested that raspberry leaf required further investigation, noting that both human and animal *in vitro* and *in vivo* data were available. The main areas of concern included general toxicity to the mother, effects on the development of the fetus or embryo and possible interactions with drugs. Others included potential transgenerational effects and effects on uterine contractility.

5. Based on the COT's recommendations, a more extensive literature search was undertaken to evaluate the safety of raspberry leaf use during pregnancy and is presented below (for full details of the search method, see Annex A).

Background

6. Leaves of the red raspberry plant (*Rubus idaeus*) have been used medicinally in Europe since as early as the 6th century (Beckett *et al*., 1954). The plant is native to Europe, North America and temperate Asia. However, it is mainly grown for commercial use in central and eastern Europe, especially Bulgaria, Macedonia and Romania (European Medicines Agency (EMA), 2013)

7. Traditionally, raspberry leaf has been recommended for a range of applications, including relieving menstrual cramps and diarrhoea, as an astringent mouthwash and as a treatment for conjunctivitis (EMA, 2013). However, it is most commonly consumed during pregnancy to stimulate and facilitate labour and to shorten its duration, with a prevalence of use among

pregnant women of 6-58 %. Typically, it is taken as tea or tablets but occasionally as a tincture (Simpson *et al.*, 2001). Other alleged benefits of raspberry leaf during pregnancy include: alleviation of morning sickness; prevention of post-partum haemorrhage, miscarriage and Braxton Hicks contractions; and stimulation of breast milk production (Patel, Rojas-Vera and Dacke, 2004; EMA, 2013).

8. Despite its long history of use, limited research has been undertaken to investigate the safety, efficacy or mechanism of action of raspberry leaf (Bowman *et al.*, 2021). Therefore, there are no health-based guidance values (HBGVs) for raspberry leaf use during pregnancy. It is also unclear what its active constituents might be. However, it is known to contain a range of different components (EMA, 2013).

9. Two of the main groups of chemicals in raspberry leaf include: hydrolysable tannins (2.6-6.9 % w/w), such as gallotannins, dimeric and tetrameric ellagitannins; and flavonoids (0.46-1.05 % w/w), such as kaempferol, quercetin and quercetin glycosides (Gudej and Tomczyk, 2004; EMA, 2013). Other components include: small quantities of volatile compounds, such as octanol; terpenoids, such as terpinolene; vitamins C and E; minerals, such as calcium, magnesium and zinc; and phenolic acids, such as caffeine and chlorogenic acid (EMA, 2013).

10. The EMA (2013) conducted a detailed literature review on the safety and efficacy of raspberry leaf by searching PubMed, Toxline, SciFinder and Cochrane Library for relevant articles published from inception until Sept 2012. The search was not restricted by language and was complemented with reference searches of extracted papers. Altogether, 499 references were identified, with those of relevance having been included in the present discussion paper.

11. In their review, the EMA (2013) highlighted that while clinical studies had not found a higher incidence of adverse pregnancy outcomes associated

with raspberry leaf treatment, treatment durations had generally been short and only a small number of pregnant women were included in the trials. It was also highlighted that there were insufficient data on genotoxicity, carcinogenicity, reproductive and developmental toxicity.

12. Due to a lack of mutagenicity (Ames test) data, the EMA (2013) could not recommend adding raspberry leaf to the Community list of herbal substances, herbal preparations and combinations thereof for traditional medicinal products. Nor did it consider the data on clinical efficacy robust enough to meet the criteria for 'well-established medicinal use,' in accordance with Directive 2001/83/EC.

13. Overall, the EMA (2013) concluded that the evidence regarding the efficacy and safety of raspberry leaf during pregnancy and lactation was lacking and that raspberry leaf could not be recommended for pregnant or lactating women, or in children and adolescents under 18.

Mechanism of Action

In Vitro and Animal Studies

14. The mechanism by which raspberry leaf may exert its alleged therapeutic effects during pregnancy is poorly understood (Bowman *et al.*, 2021). Studies seeking to address this, described below, have tended to focus on its effects upon uterine and other types of smooth muscle. However, results from these studies have been contradictory, with some reporting a stimulatory effect and others reporting a relaxant effect. These differences have been attributed to differences in raspberry leaf preparations, dosages, extraction methods, animal tissue, the pregnancy status of the uterus/uterine tissue, baseline muscle tone, and whether the raspberry leaf was tested *in vitro* or *in vivo* (Bowman *et al.*, 2021).

15. Burn and Withell (1941) conducted a series of experiments to evaluate the effects of different raspberry leaf extracts on the uterine muscle of cats and rabbits *in vivo.* They also measured the effects of the extracts *in vitro* on the uterine muscle of cats, dogs, rabbits and guinea pigs, as well as rabbit intestine (although limited information was given about the extract doses, number or types of animals used or the methods used to measure muscle tone in each case). Four extracts were prepared (**Table 1**).

Table 1: Raspberry Leaf Extraction Methods (Burn and Withell, 1941)

Method	**Description**
a).	An infusion, made by adding 10 g dried raspberry leaves to 100 ml boiling water, standing for half an hour, before filtering through a muslin. The infusion was then concentrated by evaporation at 40°C to produce an extract containing 2 g leaf/ml.
b).	An infusion prepared as in a), evaporated to dryness. The residue was mixed with 5 ml distilled water and 5 ml absolute alcohol to produce a precipitate that was removed by filtration and evaporated to dryness (20 g leaf produced approximately 1 g of residue).
c).	An infusion prepared as in a) (prior to concentration) before adding just enough lead acetate to throw down all precipitable matter and evaporating the filtrate to dryness.
d).	An infusion prepared as in a) (before concentration), treated with Norit charcoal, before being evaporated to dryness.

16. When each extract, equivalent to 2 g raspberry leaf, was injected into the jugular vein of virgin spinal cats, a 3-phasic response was observed in the uterine muscle *in vivo*: relaxation, followed by contraction and further relaxation (Burn and Withell, 1941). Occasionally, only the first or second phases were observed. These responses were usually only observed after successive injections, with the fifth injection resulting in total uterine relaxation. Conversely, when parous rabbits were injected with extract, a short-duration uterine contraction was observed. The authors suggested that this difference in response was due to the uteri of the parous rabbits having been thicker than those of the cats, which may have required a much larger dose of extract to cause relaxation.

17. A relaxant effect was observed when Burn and Withell (1941) tested the effect of the raspberry leaf extracts on non-pregnant, multi-parous cats *in vivo* and a single cat in late pregnancy. According to the authors: ‘the record obtained before the injection showed large contractions at intervals of 2-3 min superimposed on a regular rhythm of smaller contractions.’ It is unclear whether this was in reference to the non-pregnant cats or specifically to the pregnant cat. However, after the extracts were injected, ‘the large contractions were abolished during 10 min, while the series of smaller contractions persisted.’

18. The exacts produced different effects on uterine smooth muscle *in vitro*, depending on the species it belonged to and whether the muscle was in tone when the extracts were added (Burn and Withell, 1941). When the cat and dog uteri were suspended, they tended to be tonically contracted initially but relaxed after addition of extract. However, if the cat and dog uteri had been suspended for some time and had relaxed, addition of extract caused them to contract; restoring the tone of the cat uteri by adding pituitary (posterior lobe) extract to the bath, followed by raspberry leaf extract, once more caused them to relax. Conversely, the isolated rabbit uteri had little tone when first suspended but contracted following addition of the extracts. If they were contracted by addition of adrenaline, adding raspberry leaf extract caused them to relax. A relaxant effect was also observed when the extracts were tested on isolated rabbit intestine. This contrasted with the isolated guinea pig uteri, in which the only effect observed in response to the raspberry leaf extracts was contraction.

19. Burn and Withell (1941) concluded that: ‘dried raspberry leaves [contained] a principle readily extracted with water which [relaxed] the smooth muscle of the uterus and intestine when this [was] in tone,’ both *in vitro* and *in vivo* in cats. They added that: ‘the same principle or another [caused] contraction of the uterus of the rabbit [*in vivo*], and also of the isolated uterus of the cat, the rabbit and the guineapig when these [were] not in tone.’

20. Beckett *et al*. (1954) separated an aqueous raspberry leaf extract into different fractions and tested them *in vitro* for their effects on virgin guinea pig uterus and ileum, frog rectum and rat nerve diaphragm. They also tested them in terms of their *in vivo* effects on mice, chicks and chloralose-anaesthetised cats.

21. A 'purified' extract was prepared, containing the equivalent of 1 g powdered leaf/ml, by infusing dried raspberry leaves in boiling distilled water (Beckett *et al*., 1954). The infusion was strained through a muslin and a strong solution of lead subacetate added until no further precipitate was obtained. The suspension was stirred and filtered and the filtrate evaporated under reduced pressure at a temperature 'not exceeding 50°C.' It was then titrated with sulphuric acid to pH 2.8 to precipitate the calcium and excess lead present. Following filtration, the filtrate was adjusted to pH 5-6 and evaporated under reduced pressure at a temperature 'not exceeding 50°C.' Ethanol was added before the preparation was filtered again. The filtrate was evaporated under reduced pressure at a temperature 'not exceeding 30°C' to give a yellow solution which was used in all subsequent experiments. 'Purified,' pH-adjusted (pH 5-6) extract was added to a column containing a cationic exchange resin (Amberlite IR.120) and separated into various fractions using different eluants (water, N-sulphuric acid or ethanolic sulphuric acid).

22. It was noted that 'extraction [of raspberry leaf] with organic solvents yielded inactive extracts' (no further information was provided) (Beckett *et al*., 1954). However, 3 main fraction-types were isolated from the aqueous extract with activity towards isolated guinea pig ileum and uterus. These included: 'smooth muscle stimulant A,' fractions (eluted with water); 'smooth muscle stimulant B' fractions (eluted with water); and 'spasmolytic C' fractions (eluted with N-sulphuric acid or ethanolic sulphuric acid).

23. ‘Smooth muscle stimulant A’ fractions promoted contraction of isolated guinea pig uterus and ileum (doses not provided) (Beckett *et al*., 1954). When injected intraperitoneally at doses equivalent to 0.1 g leaf, these fractions also caused death following convulsions in chicks and extreme cyanosis and dilated hearts in mice. These effects were completely or partially lost when the fractions were rendered alkaline using sodium bicarbonate. Some of the alkaline solutions also started to exhibit non-specific spasmolytic action towards isolated guinea pig tissues, which was described as 'almost equally antihistaminic and parasympatholytic’ (no further information was provided).

24. ‘Smooth muscle stimulant B’ fractions caused contraction of isolated guinea pig uterus and ileum suspended in 20 ml tissue baths following addition of 0.1-1.0 ml solution (Beckett *et al*., 1954). The stimulatory effect seen towards guinea pig uterus remained the same, even after the fractions were boiled for a few minutes in sodium bicarbonate solution. They failed to potentiate acetylcholine activity, but their effects were blocked by a dose of atropine sufficient to block the action of acetylcholine (doses not provided). The stimulatory effect towards isolated guinea pig ileum occurred when 0.1 ml of each fraction was added to each 20 ml tissue bath. The contractile response was not blocked when 20 µg nicotine was added, a dose sufficient – according to the authors, to paralyse the gut’s response to nicotine. Nor was it stopped when 0.2 or 0.3 µg acetylcholine was added – a dose, the authors stated, sufficient to abolish acetylcholine activity.

25. The stimulant B fractions were further tested on isolated frog rectus suspended in 5 ml baths (Beckett *et al*., 1954). They did not produce contraction at a dose of 0.1 ml/5 ml tissue bath. However, they gradually and reversibly potentiated the stimulatory effect of acetylcholine when this was added to the bath in 1 µg doses at 5-minute intervals. The authors commented that the results provided evidence of an anticholinesterase within these fractions. This component appeared to be a stronger inhibitor of ‘pseudo-,’ as opposed to ‘true’ cholinesterases, as 0.1-0.2 ml each stimulant B fraction failed to protect acetylcholine from the ‘true’ esterase of red blood

cells; when 1.5 µg acetylcholine was added to isolated guinea pig ileum suspended in a 15 ml tissue bath containing the stimulant B fraction and 0.03 ml packed human red cells, no stimulatory response was observed compared with an untreated control. However, the stimulant B fractions effectively protected acetylcholine from destruction by plasma pseudo-esterases; when 1.5 µg acetylcholine was added to the baths containing the stimulant B fraction, a stimulatory response was observed still following pre-treatment with 0.03 ml fresh human plasma, compared with an untreated control. The weak inhibitory effect of the anticholinesterase towards the 'true' esterase was additionally supported by its failure to block the effects of curare on a rat nerve diaphragm preparation (no further information was provided).

26. 'Spasmolytic C' fractions produced a non-specific spasmolytic effect on the isolated guinea pig tissues (Beckett *et al*., 1954). When the fractions were added to 20 ml tissue baths at doses of 0.1 ml/20 ml, their spasmolytic effects antagonised the stimulatory effects of 20 µg nicotine, 2 µg acetylcholine and 1 µg histamine. The fractions also antagonised the smooth muscle stimulant actions of the other fractions (doses not provided). It was commented that: 'spasmolytic action [occurred] in the whole animal only in doses far in excess of those which [produced] nicotine-like, and muscarine-like effects' (no further information was provided)

27. In a chloralosed cat weighing 2.5 kg, the 'spasmolytic C' fractions produced muscarinic effects when 1-2 ml of each was injected intravenously – including bradycardia and a drop in blood pressure, which were abolished by injection of 2 mg atropine (Beckett *et al*., 1954). When 2-3 ml of the spasmolytic C fractions were intravenously injected into another chloralosed cat weighing 1.5 kg, which had been injected beforehand with 1.5 µg atropine, they also produced nicotinic effects – as indicated by contraction of the nictitating membrane, rise in blood pressure, tachycardia and increased respiration. Injection of 5 mg hexamethonium bromide entirely abolished these nicotine-like effects. It was suggested that this revealed a vasodepressor action, which was unaffected by 2 mg atropine or 0.5 µg

histamine. The authors explained that the nicotine-like action of these fractions was further supported by their ability to promote contraction of the frog rectus *in vitro*, which was antagonised by hexamethonium ions (no further information was provided). They added that in the 'whole animal,' larger doses of the fractions caused transient reductions in conduction through the superior cervical ganglion, following initial ganglionic stimulation (no further information was provided).

28. The authors concluded that aqueous raspberry leaf extracts contained 3 main active constituents (Beckett *et al*., 1954). The first was a smooth muscle stimulant. The second was an anti-cholinesterase. The third was a 'spasmolytic.' Therefore, the authors deduced that partially purified raspberry leaf extracts may exhibit stimulatory or 'spasmolytic' effects on isolated tissues and that previous studies had likely reported the 'mean pharmacological actions' (not defined) of complex and variable mixtures. They explained that in their study, 'the mean effect of crude raspberry leaf extracts…[was] stimulation of isolated tissues.' They further concluded that: 'it [was] rather difficult to assess the value of the traditional use of raspberry leaf infusions to give easy and speedy parturition in terms of the various active constituents exhibiting mutually antagonistic actions which [were] reported in [their] preliminary investigation.' They considered that at the time, this made it impossible to predict what the overall clinical effects would be.

29. Bamford, Percival and Tothill (1970) observed that an aqueous raspberry leaf infusion produced different effects on pregnant and non-pregnant human or rat uteri *in vitro*. The infusion was prepared by steeping 1 g dried raspberry leaf in 15 ml saline for 10 minutes at 95°C (although no other information was provided about the dose used in each experiment). When applied to pregnant rat uteri, the infusion inhibited contractions for 3-4 minutes. However, no effect was observed on non-pregnant rat uteri.

30. When the aqueous raspberry leaf infusion was tested on normal human uteri *in vitro* at 10-16 weeks of pregnancy, it caused them to contract

for ‘a few minutes’ (Bamford, Percival and Tothill, 1970). No effect was observed on non-pregnant human uteri. However, strips from normal, non-pregnant human uteri were unavailable for comparison (further details of the uterine pathology were not provided). In pregnant human and rat uteri, the intrinsic rhythm observed over a 20-minute period, while they were in contact with the infusion, became more regular and the contractions less frequent. The authors concluded that raspberry leaf may ‘modify the course of labour favourably by producing more coordinated uterine contractions.’

31. Rojas-Vera, Patel and Dacke (2002) tested different raspberry leaf extracts for their relaxant effects on transmurally stimulated guinea pig ileum *in vitro*. Extracts were prepared from 2 g samples of dried leaves using different solvents (*n*-hexane, ethyl acetate, chloroform and methanol). Each guinea pig ileum was suspended in Tyrode’s solution with 1 g tension applied and stimulated with square waves at intervals of 0.3 ms, with a supramaximal voltage of 100 V (frequency 1 Hz). The tissue was exposed to the extracts for 5-10 minutes and the relaxant activity measured by comparing average contractile force 5 minutes before extract addition to 5 minutes after.

32. The methanolic raspberry leaf extract produced the strongest relaxant effect (>80 %), while extracts prepared with less polar solvents (*n*-hexane and ethyl acetate) had no effect (Rojas-Vera, Patel and Dacke, 2002). The methanolic extract, previously extracted with *n*-hexane to remove inactive non-polar impurities, was fractionated on a silica gel column and the fractions tested individually. Two bands were identified which produced a relaxant effect on isolated guinea pig ileum. Dose-response relationships were obtained for the most active of the fractions in each band, with half-maximal inhibitory concentrations (IC_{50}) of 0.76 and 2.70 mg, respectively. However, the fraction components were not identified. The authors concluded that: ‘there [were] at least [2] chemically distinctive components displaying relaxant activity in extracts of raspberry leaves.’

33. Zheng *et al.* (2010) evaluated the effect of different commercial raspberry leaf preparations on the *in vitro* contractility of uteri from pregnant and non-pregnant nulliparous Wistar rats. The non-pregnant rats were pre-treated with diethylstilbestrol (DES) 2 days prior to sacrifice to produce an oestrogen-dominant state and the uteri of pregnant rats collected on day 20 of gestation. The commercial raspberry leaf preparations included tea, capsules or ethanolic extract (35-40 % ethanol extract). The tea and capsules were both prepared by adding the contents of the teabag or capsule to boiling, deionised water to achieve a concentration of 0.2 g/ml, before centrifuging to obtain the supernatant, which was used in the assays.

34. The effect of cumulative doses of each commercial raspberry leaf preparation was tested on isolated uterine strips from DES-treated, non-pregnant rats (1 µg/ml-4.6 mg/ml tea or capsule preparation or 2.2 ng/ml-10.1 µg/ml ethanolic extract) (Zheng *et al.*, 2010). Both the tea and capsule preparation produced uterine contractions, while the ethanolic extract had no effect. During the study, only the tea (1 µg/ml-4.6 mg/ml) was assessed for its effects on pregnant uteri and the authors commented that it excited uterine contractions to a similar extent as oxytocin.

35. The same cumulative doses of each commercial preparation were tested for their ability to affect contractions in isolated rat uteri pre-treated with 1 nmol/L oxytocin (Zheng *et al.*, 2010). In uteri from DES-treated, non-pregnant rats, none of the commercial extracts affected oxytocin-induced contractions, except at the highest concentrations (at which uterine contractions were partially inhibited). Contrastingly, in uteri isolated from pregnant animals, the tea promoted oxytocin-induced contractions in some animals and produced a biphasic response in others (excitation, followed by inhibition).

36. Zheng *et al*. (2010) also measured the effects on uterine contractility of cumulative oxytocin doses (10 pmol/L-0.15 mmol/L) added to rat uterine strips *in vitro*, following raspberry leaf pre-treatment (up to 50 minutes of exposure

to 3.2 mg/ml tea or capsule preparation, or up to 15 minutes of exposure to 6.9 µg/ml ethanolic extract). In DES-treated non-pregnant animals, oxytocin response was unaffected by pre-treatment with any of the commercial raspberry leaf preparations. Similarly, pre-treatment with commercial raspberry leaf tea had no effect on oxytocin-induced contractions in pregnant rat uteri.

37. Zheng *et al*. (2010) highlighted that each raspberry leaf preparation only produced a response at the highest concentration tested. They suggested that such concentrations were unlikely to be reached in human plasma via the oral route, due to the limited bioavailability of phytochemicals. Therefore, the authors concluded that: 'the biological activity of [raspberry leaf varied] depending on the herbal preparation used and pregnancy status'…and that the results consequently '[did] not support the hypothesis that [it augmented] labour by a direct effect on uterine contractility.' However, as Bowman *et al*. (2021) pointed out in their systematic review on raspberry leaf use during pregnancy, this assessment was based on a single serving of 1 cup of raspberry leaf tea per day, which was less than some recommendations, and did not stipulate the duration of use.

38. Olson and DeGolier (2016) tested the effects of aqueous raspberry leaf extracts on strips of mouse uterine muscle suspended in standard 15 ml organ baths. The extracts were prepared by dissolving 600 mg red raspberry leaf capsules containing 100 % red raspberry standardised extract (20 % ellagic acid) in deionised water to achieve the desired concentration. The uterine strips were from virgin female mice (*Mus musculus*, ICR CD-1® Outbred, Harlan Laboratories) given an intramuscular injection of DES 24 hours before each experiment.

39. At all concentrations tested (1.5-50 mg/15 ml organ bath), the raspberry leaf extracts produced contractile forces that were equal to or significantly greater than those produced by 10^{-5} mol/L acetylcholine ($p<0.005$) (Olson and DeGolier, 2016). The contraction force increased in a

dose-dependent manner but not always in linear fashion; the highest concentration applied did not necessarily produce the strongest response. Instead, peak responses were observed at 30-40 mg/15 ml organ bath.

40. In a separate *in vitro* experiment, Olson and De Golier (2016) also tested the effects of raspberry leaf (30 mg/15 ml bath) on mice uterine contractions, following pre-treatment with different agonists and antagonists. Pre-treatment with a cholinergic nicotinic antagonist (10^{-5} mol/L hexamethonium or curare) or muscarinic receptor antagonist (10^{-5} mol/L atropine or scopolamine) for 5-10 minutes did not inhibit raspberry leaf-induced contractions. However, pre-treatment with a β2 adrenergic agonist (10^{-5} mol/L aqueous salbutamol) inhibited any contractile response produced by the same dose of raspberry leaf. The inhibitory effects of the β2 adrenergic agonist were blocked, in turn, by addition of a β2 antagonist (10^{-5} mol/L ethanolic propranolol). Raspberry leaf-induced contractile responses were also inhibited by up to 90 %, following addition of the L-type Ca^{2+} channel blocker nifedipine (dissolved in dimethylsulfoxide (DMSO).

41. Olson and DeGolier (2016) concluded that their results supported the traditional use of raspberry leaf as a uterotonic and suggested that its uterotonic properties: '[were] not mediated through the activation of cholinergic receptors…[could] be masked or inhibited in the presence of β2 adrenergic agonist, and [were] directly or indirectly mediated through the modification of calcium channels.'

42. More recently, Hastings-Tolsma *et al*. (2022) conducted an *in vivo* study to determine the effect of raspberry leaf consumption during gestation on C57BL/6N Tac mice and their offspring (the results of which are discussed later in paragraphs 65-72). During the study, they suggested that raspberry leaf's role in promoting parturition may be related to: its inflammatory, vasodilatory and antioxidant effects; its ability to promote apoptosis in cervical and myometrial cells; and the effects of the isoflavone component genistein. The authors cited several studies to support this. During an *in vitro* study of

rabbit aortic rings, the raspberry leaf components lambertianin C and sanguiin H-6 were both shown to promote vasodilation (Mullen *et al.*, 2002). Equally, when CD-1 mice were fed diets containing 500 and 1000 ppm genistein *ad libitum* for 30 days, they exhibited significant reductions in the length of gestation compared with untreated controls (although mice fed diets containing 300 ppm genistein *ad libitum* for 150 days exhibited significant *increases* in the length of gestation) (Patel *et al.*, 2017). Similarly, an *in vitro* study by Moroney *et al*. (1988) showed that high-dose flavonoids (found in raspberry leaf) may stimulate arachidonic acid-dependent proteins, such as lipoxygenase – involved in producing inflammatory cytokines. Hastings-Tolsma *et al*. (2022) suggested that this might include prostaglandin, which is involved in cervical ripening and stimulation of uterine contractions.

43. Besides the above studies, limited and at times contradictory evidence was given to support the authors' claims about raspberry leaf's ability to promote parturition via inflammatory, vasodilatory or antioxidant mechanisms. Specifically, Hastings-Tolsma *et al*. (2022) claimed that quercetin may be one of the components in red raspberry promoting inflammatory gene expression, based on an *in vivo* study in rats by Liu, Zhang and Lu (2012). During the study, Wistar rats were randomised to receive a subcutaneous injection of either isoproterenol (70 mg/kg/body weight (bw)/day) - used to study the effects of drugs on myocardial ischaemic disorders, or isoproterenol and quercetin (70 mg/kg/bw/day and 50-150 mg/kg/bw/day, respectively). However, rather than producing inflammatory effects, as suggested by Hastings-Tolsma *et al*. (2022), in rats with immunity function impairment induced by isoproterenol injection, quercetin appeared to produce a significant reduction in inflammatory cytokines, such as IL-1, and a significant increase in anti-inflammatory cytokines, such as IL-10, compared with rats given isoproterenol only (Liu, Zhang and Lu, 2012). The authors attributed these effects, at least in part, to the antioxidant effects of quercetin. Hastings-Tolsma *et al*. (2022) also claimed that kaempferol and other raspberry leaf components exhibited pro-apoptotic effects. However, this was based on

numerous studies cited that were conducted on cancer cells *in vitro* (reviewed in Chen and Chen, 2013).

Human Studies

44. The earliest human study to examine the effects of raspberry leaf on uterine contractions was a case series published by Whitehouse (1941). Intrauterine bags were inflated inside 3 post-partum women and used to measure the effects on uterine contractions from days 5-8 post-partum following a single oral dose of: '40 grains of crude extract of dried raspberry leaves,' (2.59 g) in 1 case; '20 grains' in another (1.30 g); and 'raspberry leaf tea 20 oz. 5 %' in the third case. The woman given raspberry leaf tea was also given 5 units of pituitrin beforehand to promote uterine contractions. In each case, a relaxant effect was observed, with a reduction in the strength and frequency of uterine contractions and elimination of secondary contractions. In the woman given '40 grains of crude extract,' contractions ceased altogether. It was concluded that: 'the main effect [of raspberry leaf was] relaxation of the uterine muscle.'

Toxicity Studies

In Vitro and Animal Studies

Acute Toxicity

45. Burn and Withell (1941) prepared an aqueous raspberry leaf extract using method b). in Table 1. When mice weighing approximately 20 g were given 0.5 ml extract orally, containing the equivalent of 2 g extract, no adverse effects were observed. However, when injected intravenously, the same preparation caused convulsions and death, with a mean lethal dose of 0.1 ml/20 g mouse, corresponding to 0.4 g raspberry leaf. Similar seizures were observed after injecting cats intravenously with extracts prepared using method a). in Table 1. No additional information was provided in each case

about the dose administered or the number or types of animals used in the study.

46. Beckett *et al*. (1954) separated an aqueous raspberry leaf extract into different fractions using a column method, as described in paragraph 21. Some caused contraction of isolated guinea pig uterus and ileum (the 'smooth muscle stimulant A fractions'). When injected intraperitoneally into mice and chicks at a dose equivalent to 0.1 g leaf, these fractions acted as a central nervous stimulant and cardiovascular toxin, causing cyanosis and heart dilation in mice, and death following convulsions in chicks.

Subacute Toxicity

47. Yang *et al*. (2019) evaluated the safety of raspberry leaf extracts and powders in mice administered by gavage. Pathogen-free, 8-week-old ICR male mice were randomised to 1 of 4 groups (n=10 per group), given either: physiological saline; raspberry leaf powder (RLP); a raspberry leaf extract (RLE); or a high-temperature, high-pressure raspberry leaf extract (RLE-H). The RLP was derived from raspberry leaf plants at a plantation at the North University of China, while the RLE was prepared by extracting 25 mg/ml RLP in 60 % ethanol at 360 W for 30 minutes using a JOYN-15AL Ultrasonic apparatus. The extract was then purified using an AB-8 macroporous resin. The RLE-H was prepared by subjecting some of the RLE to a high-pressure, high-temperature treatment (121°C at 0.2 MPa for 30 minutes). The RLP, RLE and RLE-H were administered daily over 2 weeks at a dose of 100 mg/kg bw/day raspberry leaf powder. They contained approximately 15, 50 and 55 % gallic acid-equivalent polyphenols, respectively.

48. None of the mice given RLP, RLE or RLE-H died or exhibited visible signs of disease over the 2-week study period (Yang *et al.*, 2019). However, flatulence was observed in 1 of the RLE mice and 2 of the RLE-H mice, based on anatomical results. The mice also exhibited significant differences ($p<0.05$) in exhaustion time, final body weight, testes and obesity markers; after 2 weeks, the final body weight, adiposity percentage (API) and body mass index

(BMI) of the RLE and RLE-H groups decreased significantly compared with the RLP and saline-treated control groups. The testes of the RLP, RLE and RLE-H groups were significantly larger than those of the control group and the exhaustion swimming time of the RLE and RLE-H groups was significantly prolonged compared with the control and RLP groups.

49. The mechanisms behind the effects observed in the mice were not investigated (Yang *et al.*, 2019). However, the authors concluded that low doses of active phenols in the raspberry leaf preparations may modulate various physiological indexes, while high doses, such as those in the RLE and RLE-H, may lower body weight gain and API, increase testes weight and improve exercise ability but at the risk of flatulence.

Cytotoxicity

50. Wong *et al*. (2021) tested the *in vitro* toxicity of a '5-seeds' formulation containing raspberry leaf on human HEK 293 and Chang liver (HeLa-derivative) cells. Flow cytometry analysis was used to measure the proportion of SubG1 (apoptotic) cells and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays were used to measure cell viability, including the raspberry leaf dose required to produce 50 % growth inhibition (IC_{50}). The MTT assay relies on the ability of metabolically active cells to convert the water-soluble dye MTT into water-insoluble formazan crystals, which are dissolved in an organic solvent (in this case, DMSO) and quantified based on absorbance in order to estimate the number of viable cells (Rai *et al.*, 2018). The '5-seeds' formulation' - used widely to treat male infertility, also contained *Lycium barbarum, Cuscuta chinensis Lam, Schisandra chinensis* and *Plantago asiatica* (Wong *et al.*, 2021). The components were prepared and tested as aqueous extracts, both individually and as a formulation (1:1:1:1:1).

51. As the concentration of the raspberry leaf extract and '5-seeds' formulation were increased from 1-100 mg/ml, the percentage of viable HEK 293 and Chang liver cells decreased significantly compared with untreated

controls (p<0.05) (Wong *et al.*, 2021). Compared with untreated controls, there was also a marked increase in the percentage of SubG1 (apoptotic) Chang liver cells for both preparations as the concentration was increased from 25-100 mg/ml, and a marked increase in the percentage of SubG1 HEK 293 cells as the concentration of the '5-seeds' formulation was increased from 25-100 mg/ml (the percentage of SubG1 HEK 293 cells treated with the raspberry leaf extract was not reported). As the concentration of the formulation increased from 25-100 mg/ml, the proportion of SubG1 Chang liver cells increased from 10.17-36.80 %, while the percentage of SubG1 HEK 293 cells increased from 13.08-17.32 %. This compared with 1.72 % and 0.82 % for untreated HEK 293 and Chang liver cells, respectively. Similarly, as the concentration of raspberry leaf extract was increased from 25-100 mg/ml, the percentage of SubG1 Chang liver cells increased from 30.37-52.53 %.

52. The formulation exhibited higher IC_{50} values than the raspberry leaf extract alone when tested on HEK 293 cells (33 mg/ml vs. 21.2 mg/ml) and Chang liver cells (38.5 mg/ml vs. 20.1 mg/ml) (Wong *et al.*, 2021). The proportion of SubG1 Chang liver cells was also found to be considerably lower following treatment with 25 mg/ml formulation compared with when they were treated with an equivalent concentration of raspberry leaf extract (30.37 % vs. 10.17 %). The authors concluded that the formulation appeared to have modulated the toxicity of the individual herbs used to make it and that it was 'potentially non-toxic as a concoction to HEK 293 kidney and Chang liver cells.'

Genotoxicity and Carcinogenicity

53. No studies which had investigated the genotoxicity or carcinogenicity of raspberry leaf were found.

Reproductive and Developmental Toxicity

54. Graham and Noble (1955) evaluated the anti-gonadotrophic effects of aqueous raspberry leaf extracts on pregnant rat serum. The authors

described the study as an *in vitro* study, as the raspberry leaf extracts were mixed with the pregnant rat serum *in vitro* but they were subsequently injected into immature Sprague-Dawley rats. The extracts were prepared from dried and fresh raspberry leaves harvested in the Summer or Autumn. They were extracted 3 times with distilled water at room temperature, before being concentrated *in vacuo* at temperatures <50°C. The rats (n=2-8 per group) were subcutaneously injected with a 0.5 ml mixture, prepared by combining 100 I.U. μg^{-1} pregnant rat serum dissolved in 0.1 ml water with 0.4 ml raspberry leaf extract. Control rats were given an equivalent dose of pregnant rat serum but without the raspberry leaf extract. The animals were autopsied 3 days later and their ovaries weighed in order to determine the extent to which different raspberry leaf doses had inactivated the pregnant rat serum *in vitro*. The doses described below were expressed as a dried equivalent.

55. Although statistical significance was assessed using the Fisher's 't' test, *p*-values were not provided (Graham and Noble, 1955). However, given that the mean ovarian weight of the control rats was 149 mg, while that of the rats given a 10-18 mg dose of fresh or dried raspberry leaf extract ranged from 20-92 mg, there was a marked reduction in ovarian weight compared with the control group. The ovarian weight of rats given 0.8-4 mg of each extract was also considerably lower than that of the control group, at 28 mg (fresh extract from leaves belonging to old shoots), 60 mg (fresh extract from leaves belonging to new shoots), 34 mg (extract from dried leaves assayed 8 months after picking in the Summer) and 74 mg (extract from dried leaves assayed 15 months after picking in the Summer). The authors concluded that raspberry leaf 'possessed an appreciable amount of [*in vitro* anti-gonadotrophic] activity.'

56. Johnson *et al*. (2009) evaluated the effects of raspberry leaf consumption in nulliparous female Wistar rats and their female offspring. The rats (n=10 per group) were randomised to receive either: vehicle (flavoured gelatine); 10 mg/kg bw/day of a commercially available raspberry leaf product (no further information was provided); or an equivalent dose of raspberry leaf

flavonoids (kaempferol or quercetin). Each were administered orally from the point breeding was confirmed, based on the presence of sperm in a vaginal swab, to parturition. Pregnancy outcomes in the pregnant rats (P generation) were assessed, along with fertility in the first (F1) generation female offspring.

Pregnancy outcomes included: gestation length; litter size; total litter weight; birth weight; pregnancy success rate (percentage of dams with a confirmed mating which delivered a live litter); live birth index (percentage of offspring delivered that were live); sex ratio; litter survival to postnatal day 4; survival to weaning at postnatal day 21; and puberty onset, based on a visual check for vaginal opening at postnatal day 21 (Johnson *et al.*, 2009). Fertility was assessed at 6 months in the F1 dams, based on: time to pregnancy, as confirmed by detection of sperm in a vaginal swab; gestation length; mating success rate (percentage of females mated of those which cohabited); pregnancy success rate; live birth index; litter size; birth weight; total litter weight; sex ratio; and litter survival to postnatal day 4 and weaning.

57. Raspberry leaf use during pregnancy was associated with a significant increase in the length of gestation compared with the control group given the vehicle only (p<0.05) (Johnson *et al.*, 2009). There was a trend towards lower pregnancy success rate among the raspberry leaf and quercetin treatment groups, compared with the control group (78 and 90 % vs. 100 %, respectively). However, these differences were not statistically significant. Compared with the control group, dams given quercetin had significantly increased weight gain during pregnancy, while offspring exposed to raspberry leaf *in utero* exhibited a significant reduction in time to vaginal opening (p<0.05). Johnson *et al*. (2009) suggested that the latter may have been related to kaempferol in the raspberry leaf, which has been shown to exhibit oestrogenic effects on MCF7 breast cancer cells *in vitro* (Yoshikawa *et al.*, 2000). However, when Johnson *et al*. (2009) tested kaempferol in isolation, they observed no effect on time to vaginal opening.

58. Except for quercetin and kaempferol, which had no effect on any other pregnancy or birth outcomes, Johnson *et al*. (2009) suggested that the reduction in pregnancy success rate seen in dams exposed to raspberry leaf may have been related to the effects of flavonoids. Several *in vitro* studies of human and animal sperm have shown that flavonoids may adversely affect sperm activation, motility and viability at doses as low as 10 nmol/L, compared with untreated controls (Li *et al.*, 1997; Khanduja, Verma and Bhardwaj, 2001; Fraser *et al.*, 2006). The isolated flavonoid apigenin has also been shown to significantly inhibit implantation in Wistar rats following oral administration of 5-25 mg/kg bw/day (Hiremath *et al.*, 2000), compared with untreated controls, while subcutaneous administration of the flavonoid daidzein has been shown to significantly inhibit implantation in Sprague-Dawley rats at doses of 150 mg/kg bw/day (Wu *et al.*, 2005).

59. While the average birth weight of the second generation (F2) pups did not differ significantly between raspberry leaf-exposed and control dams, a significantly higher proportion of their offspring were growth-restricted (possessing a birth weight more than 2 standard deviations lower than the mean birth weight of the control offspring) (p<0.02) (Johnson *et al.*, 2009). The authors noted that in humans, growth restriction is an established risk factor for obesity, type 2 diabetes and hypertension in adulthood (Barker, 2004). For all other outcomes, maternal or fetal raspberry leaf exposure had no significant effect. Johnson *et al*. (2009) concluded that: ‘in Wistar rats, exposure to raspberry leaf extract throughout pregnancy [increased] gestation length and [resulted] in altered reproductive development and function in the offspring…which [raised] concerns about the safety of this herbal preparation for use during pregnancy.’

60. Makaji *et al*. (2011) conducted a study in rats to determine whether maternal exposure to raspberry leaf and its constituents could permanently alter the biotransformation of fluorogenic substrates by cytochrome P450 (CYP) enzymes in the livers of male and female offspring. Nulliparous female Wistar rats (n=10 per group) were randomised to receive either: vehicle

(flavoured gelatine); 10 mg/kg bw/day raspberry leaf from commercially available capsules (containing 0.2-0.4 % quercetin, 0.2-0.4 % kaempferol and 2-7 % ellagic acid, based on dry weight); or 10 mg/kg bw/day kaempferol, ellagic acid or quercetin. Each treatment was administered orally from the point that breeding was confirmed, based on detection of sperm in a vaginal swab, to parturition. Biotransformation of 8 fluorogenic substrates was measured in microtiter plates at 37°C by preparing microsomes from the biopsied livers of male and female offspring sacrificed at different time points, including: birth (postnatal day 1); weaning (postnatal day 21); postnatal day 65; and postnatal day 120.

61. The fluorogenic substrates used included: 3-[2-(N,N-diethyl-N-methylammonium)ethyl]-7-methoxy-4- methylcoumarin (AMMC); 7-benzyloxy-4-(trifluoromethyl)- coumarin (BFC); 7-benzyloxyquinoline (BQ); 3-cyano-7-ethoxycoumarin (CEC); 7-methoxy-4-(trifuoromethyl)-coumarin (MFC); methoxyresorufin (MRES); ethoxyresorufin, (ERES); and 7-ethoxy-4-trifloromethyl-coumarin (EFC) (Makaji *et al.*, 2011). For ERES and MRES, product formation was monitored by measuring emission at 590 nm after excitation at 535 nm. For AMMC, product formation was monitored by measuring emission at 460 nm after excitation at 390 nm. For BFC, BQ, EFC and MFC, emission was measured at 538 nm after excitation at 410 nm, while for CEC, emission was measured at 460 nm after excitation at 410 nm (Makaji *et al.*, 2010).

62. Except for CEC and ERES, biotransformation was gender-specific in the control group given the vehicle only (Makaji *et al.*, 2011). Excluding MRES, biotransformation was higher among males compared with females. Compared with the control group, maternal consumption of raspberry leaf significantly (p<0.05) increased biotransformation of BFC, EFC and MFC but only in female offspring and only at postnatal day 120, producing a more masculine profile. Maternal quercetin consumption also significantly increased BFC, EFC and MFC biotransformation in females, as well as EFC biotransformation in male offspring but only at postnatal days 120 and 65,

respectively (p<0.05). There was no difference in the biotransformation of the other substrates in the male or female raspberry leaf-exposed offspring.

63. Only female offspring were affected by maternal kaempferol exposure but only with respect to 2 of the substrates (ECF and MFC) (Makaji *et al.*, 2011). Compared with the control group, kaempferol significantly increased EFC and MFC biotransformation in female offspring at postnatal day 120 (p<0.05). Only 2 substrates (BQ and ERES) were affected by ellagic acid exposure; in female offspring, maternal ellagic acid exposure significantly decreased ERES biotransformation at postnatal day 21, while in male offspring, it increased the biotransformation of BQ at postnatal days 65 and 120 (although the difference only reached statistical significance (p<0.05) at day 65).

64. The authors concluded that maternal consumption of either raspberry leaf or some of its constituents led to alterations in the CYP activity of male and female offspring (although the mechanisms behind this were not investigated) (Makaji *et al.*, 2011). If applicable to humans, they suggested that the effects of such 'enzyme imprinting,' associated with consuming raspberry leaf or its constituents during pregnancy, may lead to inappropriate biotransformation (culminating in treatment failures), increased activation of xenobiotics (culminating in increased tumour formation) and altered steroid hormone biotransformation (culminating in adverse reproductive health/fertility).

65. Hastings-Tolsma *et al*. (2022) performed a study to determine the effect of daily raspberry leaf consumption during gestation on pregnant C57BL/6N Tac mice and their offspring. The raspberry leaf was prepared by boiling 53.95 g fresh raspberry leaves in water for 15 minutes, filtering and sublimating the resulting solution to yield a 10.4 g dried tea extract, which was dissolved in deionised water to produce the concentrations tested in the study. Once mating was confirmed, based on the presence of a copulatory plug, the mice were randomised (n=10 per group) to receive 1 of 3 fluids orally

in water bottles, with *ad libitum* access until parturition: a placebo, consisting of plain water; a low-dose, raspberry leaf extract containing 1.78 mg/ml dried tea extract; or a high-dose, raspberry leaf extract containing 2.66 mg/ml. The average time to litter was 21 days. The water bottles were weighed each day to determine the approximate amount of fluid and extract consumed by each mouse during pregnancy. Key outcomes measured included: differences in the length of gestation, measured from the time a copulatory plug was noted until birth; gestational weight gain, measured daily from cage placement; litter size, based on viable and non-viable pup count at birth; differences in offspring weight and physical development, measured in the morning on postnatal days 4-14; righting reflex ability, measured on postnatal days 4, 5, 6 and 7; geotaxis reflex, measured on postnatal day 8; cliff avoidance reflex, measured on postnatal day 10; and swimming development, measured on postnatal day 12.

66. According to the authors, there was no significant difference in the length of gestation between each of the 3 groups, although a statistically significant *p*-value of <0.04 was stated in the text (Hastings-Tolsma *et al.*, 2022). It was also commented that there was a trend towards the high-dose group having a longer gestation than the placebo group.

67. There was no significant difference in total fluid consumption during pregnancy between each group, except for the high-dose raspberry leaf group, which consumed significantly more fluid compared with the placebo mice ($p<0.05$) (Hastings-Tolsma *et al.*, 2022). It was suggested that this may have been related to the diuretic properties of flavonoids and polyphenols in raspberry leaf, as seen during an *in vivo* study in rats by Păltinean *et al*. (2017). The study observed a significantly greater increase in urinary volumetric excretion in Crl: WI rats given *Fumaria officinalis* extract (250 mg/kg bw/day) containing high concentrations of the flavonoids rutin (854 µg/g/dried weight) and isoquercitrin (506 µg/g/dried weight), compared with untreated controls.

68. Mice given both high and low-dose raspberry leaf had a significantly smaller litter size (viable and non-viable) than the placebo group (p<0.04) (Hastings-Tolsma *et al.*, 2022). The placebo group had a mean of 8.3 viable pups, the low-dose group had a mean of 5.1 and the high-dose group a mean of 3.8. It was suggested that this may have been related to the effects of polyphenols, such as genistein. When tested in mice and rats, both *in vitro* and *in vivo*, polyphenols have been reported to adversely affect embryo implantation and fetal survival (Jefferson, Padilla-Banks and Newbold, 2005; Christiaens *et al.*, 2008; Patel *et al.*, 2017; Shahzad *et al.*, 2017). For example, Jefferson, Padilla-Banks and Newbold (2005) observed that outbred female CD-1 mice injected with 0.5 or 5.0 mg/kg bw/day genistein at neonatal days 1-5 showed statistically significant reductions in the number of live pups over time with increasing dose, compared with untreated controls. Mice treated with a higher dose of 50 mg/kg bw/day did not deliver any live pups.

69. Hastings-Tolsma *et al*. (2022) suggested that quercetin might also have contributed towards the reduction in litter size observed in the raspberry leaf treatment groups, compared with the placebo group. In 2-6-month old, nulliparous female C57BL/6 or transglutaminase 2 (TG2)-null mice given dietary quercetin (5 mg/kg/bw/day) over a 9-month period, there was an almost 70 % increase in litter size compared with controls given an equal dose of DMSO (p<0.01), although in mice given the same dose of quercetin at 8-11 months old over 9 months, there was a significant *decrease* in litter size (p<0.01) (Beazley and Nurminskaya, 2016). Therefore, Hastings-Tolsma *et al*. (2022) hypothesised, with respect to the mice in their study, that: ‘quercetin at chronic high levels adversely effected [*sic*] fecundity in a dose-related manner.’

70. Commenting on a graph in their study, Hastings-Tolsma *et al*. (2022) stated: ‘pup weights…were lower at [postnatal days 4 and 5] when comparing high-dose treatment with low-dose and placebo [p<0.05)]’, referring to the significantly lower pup weights in the high-dose group on postnatal days 4 and 5 when compared to the placebo and low-dose groups. Contrastingly, in a

later comment, the authors stated: ‘the higher weight in pups from high-dose groups [on postnatal day 5] was likely due to the fact that pups in smaller litters were able to access more food than pups from groups where there were high litter numbers.’

71. No other significant differences were observed between each group of mice with respect to the study outcomes (Hastings-Tolsma *et al.*, 2022). However, it was noted that there was a trend towards reduced neurodevelopmental behaviour in both raspberry leaf treatment groups, compared with the placebo group, especially among the high-dose group. This included a trend towards increased time to righting on postnatal days 4 and 6 in the high-dose group (conversely, there was a shorter time to righting on postnatal days 5 and 7). Similarly, geotaxis reflex exhibited a trend towards increased response time in both raspberry leaf groups (although cliff-avoidance reflex time decreased as raspberry leaf dose increased, compared with the placebo group). Based on swim tests, this was accompanied by a trend towards lower hind leg position, tail movement and swim line component scores, in addition to overall total swim score.

72. The authors concluded that when ingested throughout gestation in mice, raspberry leaf may impact length of gestation, fluid intake during pregnancy, litter size and viability, as well as pup development (Hastings-Tolsma *et al.*, 2022).

Drug-Herb Interactions

73. Langhammer and Nielsen *et al*. (2014) conducted an *in vitro* study to investigate whether different herbal products may interfere with CYP activity, including raspberry leaf. In this study, the authors tested the products for their inhibitory effect on recombinant human CYP enzymes, initiating metabolism using an NADPH regenerating solution (containing 31 mM NADP+, 66 mM glucose-6-phosphate, 66 mM aqueous $MgCl_2$) and substrate (0.6 nM phenacetin in 1.2 % ethanol, 0.1 mM dextromethorphan or 0.8 mM testosterone in 7.8 % acetonitrile). The enzymes included: CYP1A2, which is

constitutively expressed in the liver but not believed to be involved in the metabolism of orally-administered xenobiotics; CYP2D6, which is present in both the small intestine and liver; and CYP3A4, which – as the authors pointed out, is the most abundant CYP enzyme in the intestine, playing a vital role in intestinal metabolism. The authors added that inhibition of intestinal CYP3A4 enzymes may lead to clinically significant increases in the plasma concentrations of drugs such as midazolam or felodipine.

74. Ethanolic raspberry leaf extracts were prepared from the content of commercially available capsules, ranging in concentration from 4-28,000 µg/ml in 4 % ethanol, and were incubated with each of the CYP enzymes (Langhammer and Nilsen, 2014). The rate of metabolite (acetaminophen) production by CYP1A2 was measured using validated liquid chromatography mass spectrometry (LCMS/MS) and the rate of metabolite (6β-hydroxy-testosterone) production by CYP3A4 and CYP2D6 (dextrorphan) measured using high-performance liquid chromatography (HPLC). CYP activity inhibition plots were prepared and used to determine IC_{50} inhibition constants by linear regression.

75. Some of the strongest IC_{50} inhibition constants were exhibited by raspberry leaf towards CYP1A2, CYP2D6 and CYP3A4, with IC_{50} constants of 44, 47 and 81 µg/ml, respectively (Langhammer and Nilsen, 2014). Based on the recommended dosing of the different commercial herbal products (720 mg tablets, 3 times daily for raspberry leaf) and a blood volume of 5 L, the authors concluded that clinically relevant systemic CYP inhibitions could be possible for raspberry leaf, in exceedance of these IC_{50} values. Given an intestinal volume of 250 ml, they hypothesised that it may also cause clinically relevant inhibition of intestinal CYP3A4 and that the *in vivo* inhibitory potential of the herb towards specific CYP enzymes should be investigated further.

76. Holst *et al*. (2011) administered a self-completed survey of herbal remedy use during pregnancy, which looked at parallel use of other pharmaceuticals. The survey was given to 1,037 expectant mothers who were

at least 20 weeks pregnant and who presented at an antenatal clinic at Norfolk and Norwich University Hospital between 26 Nov 2007 and 15 Feb 2008. Of the 578 respondents (response rate 55.7 %), 137 (23.7 %) reported using raspberry leaf during pregnancy. There were 232 (40.1 %) respondents who reported using both herbal remedies and pharmaceuticals during pregnancy. It is unclear what the other pharmaceuticals were or whether the women experienced any adverse effects. However, the authors commented that 4 women reported simultaneous use of iron and a tannin-containing herb (raspberry leaf, chamomile or valerian).

77. Holst *et al*. (2011) added that tannin-containing supplements may interfere with iron absorption, which should be taken into account in anaemic patients. Indeed, human studies have shown that tea consumption may lower non-haem iron absorption, with polyphenolic compounds such as tannins recognised as the likely cause, due to their ability to chelate non-haem iron (Disler *et al.*, 1975; Rossander, Hallberg and Björn-Rasmussen, 1979; Lesjak and K S Srai, 2019). However, no studies were identified in the present review which had evaluated the effects of raspberry leaf tea on iron absorption. Similar observations were made by Bowman *et al*. (2021) in their systematic review. The authors searched 6 different databases for studies evaluating the safety of raspberry leaf during pregnancy published from database inception to Jan 2019, including *in vitro*, animal and human studies. The databases included CINAHL, MEDLINE, Cochrane Library, Scopus, Web of Science Core Collection and AMED. Bowman *et al*. (2021) concluded that: ‘human studies [had] not shown any harm’ associated with raspberry leaf use during pregnancy.

Human Studies

78. According to the authors of one of the animal studies described earlier, concern has been expressed in the lay press that raspberry leaf may promote miscarriage (Johnson *et al*., 2009). The exact reasons for this are unclear but the authors pointed out that: ‘these articles [were] unaccompanied by any evidence to support the claim.’ Similar comments were made by Simpson *et*

al. (2001) during the literature review accompanying their human trial of raspberry leaf during pregnancy (described below in paragraphs 84-90); it was noted that 'medical opinions are often opposed to the use of raspberry leaf, believing it may cause or augment a miscarriage or premature labor.' However, the only evidence the authors could find to support this was a study by Bamford, Percival and Tothill (1970) (described earlier in paragraphs 29 and 30), which found that aqueous raspberry leaf extracts were able to contract human uterine muscle *in vitro* at 10-16 weeks of pregnancy.

79. One of the earliest human toxicity studies on raspberry leaf was a retrospective cohort study by Parsons, Simpson and Ponton (1999). The study was conducted at hospitals in Sydney, Australia. It included a convenience sample of 57 women approached during their postnatal stay who reported using raspberry leaf during pregnancy, and a control group of 51 women randomly selected from the hospital database who stated they had not used raspberry leaf during pregnancy. The raspberry leaf users were given a questionnaire to complete, asking about the type, dosage and duration of their raspberry leaf consumption during pregnancy, as well as subjective information about their experiences of using the herb. All participants consented to their medical records being accessed. Key safety outcomes measured included: maternal blood loss at birth (>600 ml); babies' Apgar score at 5 minutes; and maternal diastolic blood pressure pre-labour.

80. The control and experimental groups were comparable in terms of weight, parity, level of obstetric care (public or private) and age, with 'no real difference' between them with respect to these variables ($p<0.05$) (Parsons, Simpson and Ponton, 1999). They were also comparable in terms of ethnicity, although no *p*-value was given for this; most were Caucasian women (94.7 % in the raspberry leaf group and 84.3 % in the control group). Women reported commencing raspberry leaf consumption, either as tea (56.1 %), tablets (40.4 %) or a combination of tea, tablets and tinctures (3.5 %), from as early as 8 weeks' gestation. Doses ranged from 1-8 cups of tea or tablets daily but only

included a single dose of tincture (no further information was provided about the doses taken or how they were prepared).

81. Altogether, 13 % of women commenced raspberry leaf use between 8 and 28 weeks, 59 % from 30-34 weeks and 28 % from 35-39 weeks (Parsons, Simpson and Ponton, 1999). The duration of consumption was over a 1-32 week continuous period. Six women reported ceasing raspberry leaf consumption during pregnancy. Of these: 2 did so because they disliked the taste; 1 because they 'took castor oil instead;' 1 because they experienced 'early labour pains' (this was the onset of full-term labour); 1 because they experienced an increase in the frequency of Braxton Hicks contractions; and 1 because they experienced an episode of diarrhoea after consuming raspberry leaf tablets for a week at 32 weeks' gestation. However, most women reported a positive experience of taking raspberry leaf during pregnancy, with >80 % stating they would consider using it in future pregnancies or would recommend it to a friend.

82. Parsons, Simpson and Ponton (1999) identified no adverse effects associated with raspberry leaf consumption, based on information from the hospital obstetric database and participants' medical records. There was no significant difference in maternal blood loss, babies' Apgar scores at 5 minutes of age, pre-labour maternal diastolic blood pressure or transfer to a special/intensive care baby unit. Nor was there any significant difference in the length of gestation, likelihood of labour augmentation, incidence of meconium liquor, need for an epidural or length of each of the 3 stages of labour. While not statistically significant, raspberry leaf users had a shorter mean duration of the first stage of labour compared with the control group. There was also a trend towards raspberry leaf users being less likely to require an artificial membrane rupture, caesarean section, forceps or vacuum birth.

83. The authors concluded that: 'the findings [suggested] that the raspberry leaf herb [could] be consumed by women during their pregnancy…to shorten

labour with no identified side effects for…women or their babies' (Parsons, Simpson and Ponton, 1999). However, the authors pointed out that the findings may have been impacted by lack of control for potential confounders, such as the amount of raspberry leaf products consumed or choice of care provider. In their systematic review, Bowman *et al*. (2021) also pointed out that the veracity of the findings may have been affected by the small sample size and by potential selection bias.

84. Simpson *et al*. (2001) performed a double-blind, randomised, placebo-controlled trial to evaluate the safety and efficacy of raspberry leaf tablets in shortening and easing labour when consumed from 32 weeks' gestation. The study was conducted at a hospital in Sydney, Australia. The sample consisted of 192, low-risk nulliparous women (mostly Caucasian) with a healthy pregnancy, who were randomised to receive either a placebo or raspberry leaf tablets containing 2.4 g extract daily with food in 2 separate 1.2 g doses (n=96 women per group). However, no further information about the raspberry leaf tablets or randomisation process was given, such as who prepared the randomised tablet bottles, as Bowman *et al*. (2021) have commented. The raspberry leaf and placebo tablets both contained calcium phosphate, cellulose microcrystalline, magnesium stearate and soy polysaccharides. There was no statistically significant difference between the raspberry leaf and placebo groups in terms of age, weight or other demographic factors. However, it was noted that more women in the raspberry leaf group chose to receive care from a private obstetrician compared with the placebo group (11.5 % vs. 5.2 %).

85. The following outcomes were used to assess the safety of raspberry leaf during pregnancy using data from the hospital obstetric database and subjects' medical records: maternal blood loss at birth (estimated visually); maternal diastolic blood pressure at first antenatal clinic presentation in early pregnancy, at 32 weeks and pre- and post-birth; presence of meconium-stained amniotic fluid; newborn Apgar score at 5 minutes; newborn birth

weight; newborn admission to neonatal intensive/special care facilities; the occurrence of participant-reported side effects (Simpson *et al.*, 2001).

86. Raspberry leaf consumption compliance in the study was high, with 89 % of tablets consumed per woman, on average (Simpson *et al.*, 2001). A total of 48 women withdrew from the study by choice or on the investigators' advice. Six experienced nausea while taking the tablets (3 of whom were taking raspberry leaf and 3 of whom were taking the placebo). One woman withdrew due to constipation and 2 (both in the placebo group) withdrew due to diarrhoea. Some were withdrawn on medical grounds, such as developing hypertension but these women were evenly distributed among both groups. Others withdrew for non-specific reasons, such as transferring to another hospital.

87. There were no side effects that could be directly attributed to the raspberry leaf, except possibly constipation, which was exclusively observed in 4 of the raspberry leaf participants (Simpson *et al.*, 2001). Common complaints, such as nausea, vomiting and diarrhoea were 'evenly distributed between the groups,' although no *p*-value was reported with respect to occurrence of side effects. The authors suggested that these effects were most likely pregnancy-related but suggested that constipation might have been related to the purported astringent properties of raspberry leaf.

88. Similarly, there were no significant differences between the raspberry leaf and placebo groups with respect to other safety outcomes, including: maternal blood loss; maternal diastolic blood pressures; newborn Apgar score at 5 minutes; presence of meconium-stained fluid; or newborn birth weight (Simpson *et al.*, 2001). The babies in the placebo group tended to have a higher average Apgar score at 5 minutes with a narrower spread of measures but this difference was not significant (p=0.108). There were 17 admissions (8.9 %) to the Neonatal Intensive Care Unit or Special Care Nursery within 24 hours of birth. Of these, 10 babies (5.2 %) were from the raspberry leaf group and 7 (3.7 %) from the placebo group. No *p*-value was provided with respect

to this outcome but the authors noted that the frequency of admissions in the raspberry leaf group was still below the average admission rate for term babies born within the participating hospital at the time of the study (5.8 %).

89. There were no significant differences reported with respect to any of the efficacy outcomes, including: length of gestation; incidence of labour induction; incidence of artificial membrane rupture; use of patient-requested pethidine and/or epidural block; length of the first, second and third stages of labour; or emergency caesarean rate (Simpson *et al.*, 2001). While not statistically significant, there was a slight reduction in the mean length of the second stage of labour (9.6 minutes), a lower rate of forceps-assisted births and a lower incidence of artificial membrane rupture (to accelerate or initiate labour) in the raspberry leaf compared with the placebo group.

90. It was concluded that the raspberry leaf dose used appeared to be safe for mother and baby, while potentially reducing the length of the second stage of labour and the need for artificial membrane rupture during labour or forceps-assisted birth (Simpson *et al.*, 2001).

91. Nordeng *et al*. (2011a) conducted a retrospective cohort study to investigate the use of herbal drugs by pregnant women in relation to concurrent use of conventional drugs, delivery and pregnancy outcome. The women were interviewed using a structured questionnaire within 5 days after delivery. To be included in the study, they had to present at the Stavanger University Hospital, Norway, during the study period (Nov 2003-Mar 2004). During the interview, the women were questioned about their use of herbal drugs – which the interviewers described to participants as ‘any remedy produced from a herb or herbs with the intent to cure or prevent illness, to alleviate symptoms or to gain better health.’ The women were also asked for information about their use of homeopathic remedies, infant birth weight, gestational age and use of pain relief or conventional medicines during pregnancy, which was cross-checked against their medical records. When conventional and herbal medicines were used concomitantly, potential drug-

herb interactions were also assessed according to the literature (Mills, 2006). The women's medical charts were reviewed with respect to several pregnancy and delivery outcomes, including: infant birth weight; gestational age; presence of neonatal and/or maternal complications after delivery; delivery mode; use of acupuncture; and use of pharmacological analgesics during delivery.

92. Of 648 women invited to partake in the study, 600 agreed (participation rate of 92.6 %) (Nordeng *et al.*, 2011a). Socio-demographic characteristics were similar among user and non-users of herbal drugs, except for educational level. A significantly higher proportion of women who reported using herbal drugs during pregnancy had tertiary education compared with non-users (59.2 % vs. 49.7 %, p=0.006). In 2 cases, women reported using 'iron-rich herbs' and acid suppressants, which the authors commented may result in a 'mutually decreased effect.' However, the category 'iron-rich herbs' was not defined and no further information was provided on their potential interaction with acid suppressants. Altogether, 34 (5.7 %) of the women reported using raspberry leaf during pregnancy – usually to prepare the uterus for labour. Information on the type of raspberry leaf consumed or the dose or timing of administration were not collected. There were no statistically significant differences between herbal drug users and non-users for any of the pregnancy or delivery outcomes evaluated, except for delivery mode and birth weight.

93. Mean birth weight was 155 g higher among herbal drug users during pregnancy (3663 g vs. 3508 g) (p=0.001) (Nordeng *et al.*, 2011a). Sub-analyses of the most commonly used herbs revealed that this was mainly related to the use of 'iron-rich herbs' (p=0.005), which they suggested may have had a positive nutritional effect on mother and child. However, since information on other factors which may have influenced infant birth weight was not collected, such as maternal smoking status or BMI, the authors could not discount the possibility of confounding.

94. With respect to delivery mode, a statistically significant association was identified between raspberry leaf tea intake during pregnancy and increased

likelihood of caesarean section – even after controlling for maternal age, parity, marital status, education and conventional drug use (23.5% vs 9.1%; adjusted odds ratio 3.47; 95% confidence interval (CI) 1.45–8.28) (Nordeng *et al.*, 2011a). This led the authors to conclude that use of raspberry leaf prior to delivery was associated with an increased risk of caesarean delivery. However, no such association was found in the 2 Australian studies looking at the effects of raspberry leaf tea taken during pregnancy, described above (Parsons, Simpson and Ponton, 1999; Simpson *et al*., 2001). Nordeng *et al*. (2011) also pointed out that the association between raspberry leaf use and likelihood of caesarean delivery could have been confounded by underlying medical conditions in the raspberry leaf group.

95. According to a case report by MacPherson and Kiliminster (2006), a 32-year old Caucasian female gave birth to a neonate with status epilepticus after she consumed a mixture of raspberry leaf, blue cohosh and squaw vine for 4 days in late pregnancy in order to accelerate labour. The pregnancy was described as otherwise 'uneventful' and the mother's health 'unremarkable.' Limited information about the herbal mixture was available. However, the patient described preparing the mixture by combining 20 ml of blue cohosh, 20 ml of raspberry leaf and 20 ml of commercially available squaw vine, which she consumed 3 times daily (in 3 ml doses) for 4 days at 41 weeks' gestation.

96. Four days after starting self-treatment with the herbal mixture, the patient gave birth to a girl weighing 3,710 g by spontaneous delivery (MacPherson and Kilminster, 2006). No other medications or analgesics were administered during labour and delivery, except for a nitrous oxide/oxygen mixture. Within 3 hours of delivery, the neonate was experiencing tonic seizures, followed by increasing agitation, respiratory distress and abnormal neurological activity, described as bizarre 'cycling movements' of the legs, associated with irregular respiration. A diagnosis of status epilepticus was made on clinical grounds and subsequently confirmed by electroencephalogram. An MRI reported normal findings, except for a subcutaneous right parietal haematoma - commonly associated with birth,

which was considered incidental. The neonate was intubated, ventilated and treated with phenobarbitone. Their symptoms subsided and they were discharged after 8 days, with oral phenobarbitone treatment continued for 4 weeks. Findings from a second electroencephalogram at 10 weeks were normal and there were no long-term complications.

97. The authors pointed out that the use of blue cohosh had been associated with neurological complications before (MacPherson and Kilminster, 2006). MacPherson and Kiliminster (2006) also highlighted that there were several active principles in blue cohosh capable of traversing the placental barrier. These included the nicotinic receptor agonist N-methylcystine (Finkel and Zarlengo, 2004). Agonists of these receptors have been shown to produce excitatory effects in human studies, such as tachycardia, coronary vasoconstriction and increased gastrointestinal motility (Jones and Lawson, 1998). Therefore, the authors considered it plausible that N-methylcystine might be epileptogenic (MacPherson and Kilminster, 2006).

98. Wedig and Whitsett (2008) published a case report of a 2,885 g white female baby born at 38 weeks' gestation to a 31 year-old, primigravida woman by spontaneous vaginal delivery, following labour induced with Pitocin. The week before delivery, the mother had taken raspberry leaf tea and 13 x 500 mg evening primrose oil capsules, vaginally and orally, believing it would help improve labour (no additional information was provided about the doses taken for raspberry tea leaf). At 17 hours of age, the infant developed diffuse ecchymoses and petechiae on their trunk, extremities and face. She was otherwise well and had normal laboratory results. There was no history of haematological abnormalities, no evidence of cranial haemorrhage from an ultrasound or blood in the urine. The infant was subsequently discharged at 3 days of age and by 5 days of age, the purpura had resolved. The authors cited several studies in humans, rats, rabbits and baboons showing reduced platelet aggregation following ingestion of evening primrose oil or its constituents, such as gamma-linoleic acid (Guivernau *et al.*, 1994; De La Cruz *et al.*, 1997; Sim and McCraw, 1977). Based on these reports, Wedig and

Whitsett (2008) concluded that the primrose oil taken by the mother during late gestation had most likely inhibited platelet aggregation in the newborn infant, resulting in the petechiae and purpura. The authors did not comment on whether the raspberry leaf may have contributed towards these effects. However, they acknowledged that 'neither raspberry leaf tea nor primrose oil [had] been systematically shown to be either safe or effective.'

99. A case report published by Cheang *et al.* (2016) described a 38-year old, nulliparous woman with gestational diabetes mellitus (GDM) who developed hypoglycaemia, requiring a lowered insulin dose, after consuming 3 cups of raspberry leaf tea for 3 days at 32 weeks' gestation. The tea was prepared from dried raspberry leaf but no other information on the dose consumed. The patient's 2-hour postprandial glucose level after taking the raspberry leaf was 52-66 mg/dL and her bedtime glucose value 48 mg/dL. Along with hypoglycaemia, she also experienced tachycardia and mild diaphoresis, which were self-treated with orange juice. The patient did not report any other changes in physical activity or dietary habits and her insulin dose had been stable 2 weeks prior to the hypoglycaemia, and was on-target.

100. The effects of the raspberry leaf on blood glucose were confirmed by resolution of the patient's symptoms after self-withdrawal and reintroduction (Cheang *et al.*, 2016). The patient continued to use the raspberry leaf tea twice-daily throughout pregnancy after her insulin was adjusted and her blood glucose was well controlled throughout the rest of the study. Fetal surveillance was conducted weekly from 32 weeks' gestation but the results appeared normal, with no evidence of uteroplacental insufficiency or abnormalities in the child's biophysical profile or ultrasonography results. Labour was augmented at 39 weeks with oxytocin, due to prolonged membrane rupture and meconium-stained amniotic fluid. Delivery was via caesarean, due to active-phase arrest during suspected chorioamnionitis (treated with intrapartum antibiotics). However, the neonate did not experience hypoglycaemia or other complications and the placental biopsy was normal, both microscopically and macroscopically. The infant was born weighing 3,490 g (75th percentile for

gestational age) and had an Apgar score of 9 and 9 at 1 and 5 minutes, respectively.

101. Cheang *et al*. (2016) pointed out that raspberry leaf belongs to the same family as blackberry leaf (*Rosaceae*), which has also been shown to have hypoglycaemic effects when orally administered to rats (Jouad, Maghrani and Eddouks, 2002; Bispo *et al.*, 2015). Based on the potential similarities between both plant species and the details of the case report, the authors concluded that raspberry leaf may lead to reduced insulin requirements in GDM. They proposed that women taking it should be made aware of this and have their blood glucose levels closely monitored.

102. A recent case report described a 53-year old woman who presented to the emergency department with a 3-day history of jaundice, nausea, abdominal bloating and occasional loose stools after using a 'liver-cleansing' compound and a nightly herbal 'sleep aid' over a month to benefit her overall wellness (Koenig, Callipari and Smereck, 2021). Although the patient was beyond typical childbearing age, she experienced acute liver injury, which could potentially be of concern in women consuming similar raspberry leaf preparations as part of the maternal diet.

103. According to the label, the liver detoxifier contained 5 vitamins and amino acids and 16 herbal ingredients, including a 'proprietary herbal-nutrient blend' of raspberry leaf, artichoke leaf, beet leaf, black radish root, bladderwrack (whole plant), phosphatidylcholine (soy), blue flag root and cleavers (aerial parts) (Koenig, Callipari and Smereck, 2021). Other notable herbal ingredients listed included 50 mg per 'serving' (not defined) scute root concentrate (*Scutelleraria baicalensis*) and 30 mg per 'serving' turmeric root (*Curcuma longa*). No information was provided about the concentration of raspberry leaf present or the total amount of liver detoxifier consumed by the patient. Contrastingly, the 'sleep aid' label listed the ingredients as valerian (0.8 % valerenic acid, 600 mg per 'serving'), melatonin (5 mg per 'serving),

gamma-aminobutyric acid (100 mg per ‘serving’) magnesium glycinate (100 mg per ‘serving’) and vitamin B_6 (10 mg per ‘serving’).

104. The patient denied using other medications, including paracetamol and other over-the-counter analgesics (Koenig, Callipari and Smereck, 2021). She had no history of past supplement use (besides occasional multivitamins), international travel, blood transfusion or intravenous drug use – nor any history of depression or other psychiatric illness. The patient also denied alcohol use in the preceding 6 weeks, before which she described her alcohol use as ‘social.’ She denied experiencing fever, sweats, weight loss, food intolerance or spontaneous bleeding and had had no known contact with infectious diseases and no ‘significant’ medical history.

105. Blood tests identified marked hyperbilirubinaemia, with liver enzyme elevations indicative of cholestatic jaundice (total bilirubin of 25.4 mg/dL, direct bilirubin 22.1 mg/DL, aspartate transaminase (AST) 164 U/L, alanine aminotransferase (ALT) 89 U/L and alkaline phosphatase (ALP) 174 U/L) (Koenig, Callipari and Smereck, 2021). Leukocytosis (14,800 cells/µL, 85 % neutrophils) and mild coagulopathy were also noted (prothrombin time of 17.0 s, partial thromboplastin time of 36.1 s and international normalised ratio of 1.4). There were signs of white and red cells in the urine, with 5 and 2 per high-powered field respectively, along with low bacterial counts, a protein level of 10 mg/dL and specific gravity of 1.006. Alcohol or paracetamol were non-detectable in the urine. Serological screens for viral infection (Hepatitis A, B and C, HIV and HSV-1 and 2) and markers of autoimmune hepatitis (anti-smooth muscle antibodies and ceruloplasmin levels) were also negative.

106. Imaging studies, along with ultrasound and abdominal MRI, revealed hepatomegaly and steatosis without biliary dilatation (Koenig, Callipari and Smereck, 2021). A liver biopsy subsequently confirmed drug-induced liver injury. The patient’s liver function abnormalities improved with discontinuation of the products and corticosteroid treatment. Approximately 7 months after

discharge, her laboratory results were 3.7 mg/dL for total bilirubin level, 34 U/L for ALT and 139 U/L for ALP. She was subsequently lost to follow-up.

107. The authors pointed out that the detoxifier and 'sleep aid' were not available for analysis so the possibility of product mislabelling or contamination with other agents, such as heavy metals, could not be discounted (Koenig, Callipari and Smereck, 2021). They did not comment specifically on the raspberry leaf in the liver detoxifier and whether this may have contributed towards the patient's liver injury. However, they highlighted several herbal ingredients in both products which may have caused hepatotoxicity based on previous case reports, either alone or in combination with other herbal ingredients. These included: valerian (Caldwell *et al.*, 1994; Cohen and Toro, 2008; Vassiliadis *et al.*, 2009); scute root (Itoh *et al.*, 1995; Hsu *et al.*, 2006; Linnebur, Rapacchietta and Vejar, 2010; Yang *et al.*, 2012; Dhanasekaran, Owens and Sanchez, 2013); and turmeric (Luber *et al.*, 2019).

108. The World Health Organisation's (WHO) Uppsala Monitoring Centre received 2 reports from the UK's national pharmacovigilance centre, based on a search conducted on 27 Sept 2011 (EMA, 2013). Limited information was provided about the first report, except that 6 different herbal preparations had been used in 'therapeutic doses.' Therefore, it is unclear what the preparations were, whether the ingredients included raspberry leaf or whether any adverse effects were observed relevant to the maternal diet. However, in the second report, raspberry leaf had been used orally for 2 months to 'precipitate labour,' initially with 1 'dose' (not defined) per week for 1 month and then 1 'dose' daily for 30 days. At 2 days of age, the newborn experienced convulsions. No additional information was provided.

109. Similarly, since its inception in 1983, the UK Teratology Information Service (UKTIS) has received 6 reports of raspberry leaf exposure during pregnancy. Five of these cases occurred following 'therapeutic' use of raspberry leaf tea or tablets (no information was available about the dose taken). At least 3 of the 5 women who took raspberry leaf therapeutically were

exposed in the third trimester and 1 in the first (no other information about the time of exposure was available for the other women). Pregnancy outcomes for these 5 women were normal, except for 1, who gave birth to a child with cerebral palsy following a delayed delivery. The 6th case occurred after a pregnant woman consumed large quantities of 400 mg raspberry leaf tablets, having mistaken them for sweets (no further information was provided about the dose taken or the time of exposure). The woman experienced nausea and diarrhoea but no pregnancy-related symptoms and delivered a normal, liveborn infant at 40 weeks.

Contaminants

110. Very few studies have investigated potential contaminants in raspberry leaf. Those that were identified are described below.

111. Sadlo *et al*. (2015) measured the levels of fungicide and insecticide residues in commercial raspberry leaf (n=10), raspberry fruit (n=20) and soil (n=10) samples using gas chromatography and compared these to the maximum residue limits (MRL) and acceptable daily intake (ADI) values for each. The study was only available as an abstract and thus offered limited information. However, cypermethrin residues (Cyperkill 25 EC) were found at low levels in raspberry leaf samples (2.58 mg/kg), even 25 days after application. The following were also detected in the raspberry leaf: boscalid (30.64 mg/kg), pyrimethanil (8.13 mg/kg) pyraclostrobin (15.82 mg/kg) and chlorpyrifos (0.15 mg/kg). The highest average daily intake was in the case of boscalid, which in raspberry leaf reached 6.63 (units not provided), corresponding to 12.18 % of the ADI. For herbal infusions (leaves and herbs), retained EU Regulation (EC) No. 396/2005 establishes an MRL for boscalid in herbal infusions (leaves and herbs) of 0.9 mg/kg. It also stipulates that chlorpyrifos, cypermethrin, pyraclostrobin and pyrimethanil should be below specified limits of detection within these products (respectively, 0.01 mg/kg, 0.1 mg/kg, 0.1 mg/kg and 0.05 mg/kg). Therefore, the pesticide levels

measured in raspberry leaf samples by Sadlo *et al*. (2015) were each above the current legal limits for herbal infusions.

112. Kowalska (2021) tested concentrations of Cd, Pb, As and Hg in 240 samples of plant material, including 3 samples of raspberry leaf weighing at least 3 kg each. They compared the levels against the limits set by the WHO, respectively, 0.3 mg/kg, 10.0 mg/kg, 5.0 mg/kg and 0.2 mg/kg. The samples were unprocessed and collected at random from farms located in the eastern part of Poland between 2015 and 2018. Prior to analysis, the raspberry leaf samples were milled and sieved through a 0.5 mm mesh, after which the moisture level of each was determined for conversion to dry matter.

113. The metal content of the samples was determined following microwave mineralisation (involving 65 % HNO_3 and stepwise microwave treatments of 400 W at 363 K, 800 W at 393 K and 1600 W at 483 K) (Kowalska, 2021). Pb, Cd and As content was assessed using an inductively-coupled plasma mass spectrometer with quadrupole mass analyser. Hg content was determined independently using a non-flame atomic spectrometry absorption technique (Hg analyser AMA 254, Altec, Czech Republic). The results were expressed in mg/kg dry matter.

114. The Cd content in the raspberry leaf was 0.345 mg/kg, which was above the WHO limit of 0.3 mg/kg (Kowalska, 2021). The Pb, As and Hg levels were all within WHO limits, at 0.330 mg/kg for Pb, below the limit of quantification of 0.1 mg/kg for As and 0.007 mg/kg for Hg. For context, retained EU Commission Regulation (EC) No. 1881/2006, as amended, establishes limits in supplements of 1.0 mg/kg for Cd, 3.0 mg/kg for Pb and 0.1 mg/kg for Hg, (although no limits are provided for As in supplements). Therefore, the metal levels which the authors measured in raspberry leaf samples were all within the legal limits currently permitted for supplements within this Regulation.

115. Veatch-Blohm *et al*. (2021) investigated the consistency of contamination within and between duplicate bottles of herbal supplements from different manufacturers, including metals, fungi and gross contaminants. This included two bottles of raspberry leaf capsules (both purchased online from Nature's Way). Metal concentrations were measured in a single capsule subsample (0.05 g) from each bottle using flame atomic absorption spectroscopy, following HNO_3 digestion, including Ni, Zn, Pb, Cu and Cr. The remaining subsamples from each capsule were then examined under a light-dissecting microscope to check for gross physical contamination. To screen for fungal contaminants, 5 g was taken from each bottle, mixed with 45 ml sterile water and the supernatant cultured on Sabouraud Dextrose Agar plates. The plates were incubated for 5-7 days and contaminants classified based on visual characteristics. Eight control plates were also inoculated with sterile water under the same conditions.

116. Pb, Ni and Cu were not detected in either of the raspberry leaf samples (Veatch-Blohm *et al.*, 2021). Cr was detected in 1 raspberry leaf sample but at levels of 0.42 µg/g. The authors pointed out that no tolerable upper intake level (TUL) had been established for Cr, due to lack of research (Singh *et al*., 2008, cited in Veatch-Blohm *et al.*, 2021). Similarly, Zn was detected in 1 sample at levels of 1.90 µg/g - the TUL being 40 mg/day, according to the authors (Institute of Medicine US Panel on Micronutrients, 2001, cited in Veatch-Blohm *et al.*, 2021). It was suggested that there may be a risk of metal overdose among those taking multiple supplements at once over a prolonged period. For context, retained EU Commission Regulation (EC) No.1881/2006, as amended, establishes a Pb limit in supplements of 3.0 mg/kg but does not establish limits for Cu, Cr, Ni or Zn. Therefore, the metal levels which the authors measured in the raspberry leaf supplements were compliant with the limits currently specified for supplements within this Regulation.

117. Both raspberry leaf samples contained *Aspergillus fumigatus*, *Microsporum* sp. and *Nocardia brasiliensis* (Veatch-Blohm *et al.*, 2021). No gross contaminants were detected in either. The authors explained that

Microsporum is known to cause infections and may be problematic in immunocompromised individuals, while the other species are 'generally safe' but may act as opportunistic pathogens in immunocompromised individuals.

Exposure Assessment

118. Raspberry leaf is typically consumed during pregnancy as tea (fresh or dried leaf preparations), tablets or tinctures (EMA, 2013; Bowman *et al.*, 2021). Different recommendations exist as to when women should commence raspberry leaf use during pregnancy; most online sources suggest taking it from the third trimester – typically as tea or tablets, while others suggest taking it from the second or even first trimester (Annex B).

119. While not specific to raspberry leaf, data from the National Diet and Nutrition Survey (NDNS) on chronic herbal and fruit tea consumption among women of childbearing age (16-49 years) may provide an indicator of raspberry leaf tea intake during pregnancy and is displayed in Tables 2a and 2b (without recipes). The data suggests that women within this age group consume a mean of 4.5 ml/kg bw/day or 16 ml/kg bw/day at the 97.5th percentile, as consumed (calculated by applying a conversion factor of 0.99 to convert tea from dry weight to as consumed). This corresponds to a mean of 0.045 g/kg/bw/day herbal or fruit tea or 0.16 g/kg/bw/day at the 97.5th percentile on a dried basis (calculated by applying a conversion factor of 0.01 to convert tea as consumed to dry weight).

120. Significantly, it is still unclear as to what the active components in raspberry leaf are (Bowman *et al.*, 2021). However, some raspberry leaf components, such as polyphenolic compounds, may vary in concentration between different raspberry leaf preparations, depending on area of cultivation, in a way which may affect their bioactivity and potentially also their toxicity (Venskutonis, Dvaranauskaite and Labokas, 2007). Nor does NDNS data capture consumption data for pregnant or lactating women and so while the data in Tables 2a and 2b is based on women of childbearing age, it may not necessarily be representative of the maternal diet. Therefore,

consumption data for raspberry leaf tea, tablets and tinctures were also estimated during each trimester based on dose recommendations from online sources and are displayed on Tables 3a and 3b.

121. Assuming that one teabag represents 2 g tea, that a single cup holds 350 ml fluid and that capsules contain 100 % dried raspberry leaf, online sources suggest that raspberry leaf consumption ranges from 350-1,750 ml/person/day for tea, 16-24 ml/person/day for tinctures and 900-2,400 mg/person/day for tablets during pregnancy, as consumed. In terms of dried weight, this corresponds to 2-10 g/person/day for tea, 0.014-0.021 g/person/day for tinctures and 0.9-2.4 g/person/day for tablets (assuming that tinctures were prepared 1:1 in terms of weight: volume and that a fresh leaf contains 77.75 % moisture, based on nettle leaf data).

122. A retrospective cohort study suggested that at least 3.5 % of women may use a combination of raspberry leaf tea, tablets and tinctures during pregnancy (Parsons, Simpson and Ponton, 1999). Therefore, consumption values were calculated for raspberry leaf during pregnancy, assuming combined use of tea, tablets and or tinctures in order to cover different 'worst-case' exposure scenarios. These values are displayed in Table 4 and were based on online dose recommendations during the third trimester, which is when raspberry leaf appears to be most commonly taken. It is apparent that the consumption level resulting from combined use of raspberry leaf tea, tablets and tinctures may reach up to 12.421 g/person/day.

Table 2a. Estimated Chronic Consumption of Herbal and Fruit Teas as Consumed (Without Recipes)*[ab]

Consumers (n)	Mean (ml/person/day)	97.5th percentile (ml/person/day)	Mean (ml/kg bw/day)	97.5th percentile (ml/kg bw/day)	Respondents in population group (n)
364	290	1100	4.5	16	2556

*Rounded to 2 significant figures

[a] Based on females aged 16-49 in NDNS years 1-11

[b] Conversion factor of 0.99 used to convert tea from dry weight to as consumed

Table 2b. Estimated Chronic Consumption of Herbal and Fruit Teas Dry Weight (Without Recipes)*[ab]

Consumers (n)	Mean (g/person/day)	97.5th percentile (g/person/day)	Mean (g/kg bw/day)	97.5th percentile (g/kg bw/day)	Respondents in population group (n)
364	2.9	11	0.045	0.16	2556

*Rounded to 2 significant figures

[a] Based on females aged 16-49 in NDNS years 1-11

[b] Conversion factor of 0.01 used to convert tea as consumed to dry weight

Table 3a. Raspberry Leaf-Containing Teas, Tinctures and Tablets Summary Table of suggested serving sizes on an as-consumed basis (Data Pooled from Online Sources in Annex B)

	Suggested serving size per day (as consumed)	Suggested serving size per day (as consumed)	Suggested serving size per day (as consumed)
Stage of pregnancy	Tea (ml) *[a]	Tincture (ml)	Capsules /tablets (mg)
1st trimester	350	N/a	N/a
2nd trimester	700	N/a	N/a
3rd trimester	350 – 1,750	16 – 24	900 – 2,400

*Calculated on the assumption that 1 teabag contains approximately 2 g of tea

[a] Calculated on the assumption that a large mug holds approximately 350 ml of tea

N/a = no sources recommending consumption

Table 3b. Raspberry Leaf-Containing Teas, Tinctures and Tablets Summary Table Based on Dry Weight of Raspberry Leaf Consumed (Data Pooled from Online Sources in Annex B)

	Suggested serving size per day (as consumed)	Suggested serving size per day (as consumed)	Suggested serving size per day (as consumed)
Stage of pregnancy	Tea (g) *	Tincture (g) [a]	Capsules /tablets (g) [b]
1st trimester	2	N/a	N/a
2nd trimester	4	N/a	N/a
3rd trimester	2-10	0.014-0.021	0.9 – 2.4

*Calculated on the assumption that 1 teabag contains approximately 2 g of tea

[a] Calculated on the assumption that the tincture was made 1:1 weight to volume ratio. Assumption that fresh leaf is 77.75 % moisture content of nettle leaves

[b] Assumption that capsules contain 100% dried raspberry leaf tea

Table 4. Combined Consumption Scenarios of Raspberry Leaf Tea (Dry Weight)

	Estimated consumption of dry raspberry leaf (g per day)	Estimated consumption of dry raspberry leaf (g per day)	Estimated consumption of dry raspberry leaf (g per day)	Estimated consumption of dry raspberry leaf (g per day)
	Tea	Tincture	Capsule	TOTAL consumed per day
3rd trimester	2-10	0.014-0.021	0.9-2.4	2.914 – 12.421
	2-10	0.014-0.021	N/a	2.014 – 10.021
	2-10	N/a	0.9-2.4	2.9 – 12.4
	N/a	0.014-0.021	0.9-2.4	0.914 – 2.421

Risk Characterisation

123. There is considerable uncertainty surrounding the safety of raspberry leaf use during pregnancy, largely due to limited data from *in vitro*, animal and human studies. No research appears to have been conducted to evaluate whether raspberry leaf might exhibit genotoxic or carcinogenic effects and the safety of raspberry leaf use during pregnancy has only been assessed in 3 human studies, which were of short duration and included limited numbers of participants (Parsons, Simpson and Ponton, 1999; Simpson *et al*., 2001; Nordeng *et al*., 2011). Furthermore, the biological effects of raspberry leaf are unclear and the identities of the active components unknown. The concentration of any active components may vary between different raspberry leaf preparations, depending on factors such as area of cultivation (as well as processing and means of preparation) (Venskutonis, Dvaranauskaite and Labokas, 2007). This could potentially result in differences in their toxicity.

124. One of the main human studies of raspberry leaf was a double-blind, placebo-controlled, randomised trial, which found no adverse effects on mother or child in nulliparous pregnant women randomised to receive 2.4 g raspberry leaf extract per day from 32 weeks' gestation (consumed as tablets in 2 separate 1.2 g doses) (Simpson *et al.*, 2001). However, limited detail was provided about the randomisation process in the study and women receiving private maternity care were overrepresented among the raspberry leaf group. Online sources also suggest that maternal raspberry leaf consumption may reach up to 10 g/day tea, in terms of dried weight, or up to 12.421 g/day when combined with tinctures and tablets. Therefore, the dose administered during the trial may not necessarily have been representative of the doses consumed during pregnancy.

125. The other 2 human safety studies carried out were observational (Simpson *et al.*, 2001; Nordeng *et al.*, 2011). These studies also did not find

any adverse effects on mother or child associated with maternal raspberry leaf consumption, although limited information was provided about the raspberry leaf preparations, including dose, timing or duration of use. One study found that raspberry leaf use was associated with a significantly increased risk of caesarean delivery (Nordeng *et al.*, 2011). However, this was not consistent with the other 2 human safety studies (Parsons, Simpson and Ponton, 1999; Simpson *et al*., 2001).

126. Case reports suggest that raspberry leaf consumption during pregnancy may be associated with adverse effects in some women, including diarrhoea (UKTIS data) and hypoglycaemia in those with GDM (Cheang *et al.*, 2016). There was also one case report of acute liver injury associated with combined use of a 'liver-cleansing' compound containing raspberry leaf and a 'sleep aid;' the authors did not comment specifically on whether the raspberry leaf might have contributed towards this but highlighted several other herbal ingredients in each product which had been associated with acute liver injury during previous case reports, such as valerian (Koenig, Callipari and Smereck, 2021). They added that they could not rule out the possibility of product mislabelling or contamination. Other case reports of maternal raspberry leaf consumption have described adverse neonatal effects, including epilepsy (MacPherson and Kilminster, 2006), petechiae and ecchymoses (Wedig and Whitsett, 2008), convulsions (EMA, 2013) and cerebral palsy (UKTIS data). However, limited information was available in these studies about the doses taken and it is uncertain whether the adverse effects described were related to raspberry leaf consumption or to other factors, such as the use of other herbal products described in several of them (MacPherson and Kilminster, 2006; Wedig and Whitsett, 2008; Koenig, Callipari and Smereck, 2021).

127. According to Johnson *et al*. (2009), it has been suggested in the lay press that raspberry leaf use during pregnancy may promote miscarriage, although the authors were not aware of any evidence provided to support this. A similar comment was made by Simpson *et al*. (2001), who pointed out that

concerns over promoting miscarriage or premature labour had led to divided medical opinions as to whether raspberry leaf should be used during pregnancy. However, the only evidence the authors could find to support such concerns was a study by Bamford, Percival and Tothill (1970) (described earlier in paragraphs 29 and 30), showing that aqueous raspberry leaf extracts could contract human uterine muscle *in vitro* at doses equivalent to 0.06 g/ml dried leaf. However, it is worth noting that opposite results were obtained by Whitehouse (1941), who observed a relaxant effect on human uterine muscle when 3 post-partum women were orally administered 1.30 g or more dried raspberry leaf extract or 20 oz. of 5 % raspberry leaf tea.

128. A range of potential adverse effects associated with raspberry leaf exposure have also been identified during *in vitro* and animal studies. Aqueous raspberry leaf extracts were able to reduce the viability of Chang liver and HEK 293 cells *in vitro* at doses of 1-100 mg/ml and to promote apoptosis in Chang liver cells at doses of 25-100 mg/ml, compared with untreated controls (Wong *et al.*, 2021). Contrastingly, in mice, no adverse effects were observed following an oral dose of aqueous raspberry leaf extract equivalent to 10 % of body weight (Burn and Withell, 1941). However, death was observed in mice following intravenous administration of 4 g/ml aqueous raspberry leaf extract (Burn and Withell, 1941) and in chicks following intraperitoneal administration of a raspberry leaf extract containing the equivalent of 0.1 g leaf (with cyanosis and heart dilation occurring in mice given an equivalent intraperitoneal dose of the same extract) (Beckett *et al*., 1954).

129. There is also evidence that raspberry leaf may exhibit anti-gonadotrophic activity. This is based on a study by Graham and Noble (1955), who observed considerably lower ovarian weights among rats subcutaneously injected with a mixture of 0.4 ml aqueous raspberry leaf extract mixed *in vitro* with 100 I.U. μg^{-1} pregnant rat serum, compared with controls (injected with an equivalent dose of pregnant rat serum without extract). Based on another *in vivo* study in rats given an oral dose of 100 mg/kg bw/day raspberry leaf

extract (containing 15-55 % gallic acid-equivalent polyphenols), other adverse effects associated with raspberry leaf consumption may include flatulence, increased testes weight, and reduced weight gain and API (Yang *et al.*, 2019).

130. Three animal studies identified transgenerational effects in mice and rats orally administered raspberry leaf extracts during pregnancy (Johnson *et al.*, 2009; Makaji *et al.*, 2011; Hastings-Tolsma *et al.*, 2022). In the first study, pregnant rats were administered 10 mg/kg/bw/day commercially available raspberry leaf extract in gelatine capsules (Johnson *et al*., 2009). Effects reported included a significant increase in the length of gestation, together with significant growth restriction of the F2 offspring, compared with untreated controls. There was also a non-statistically significant reduction in pregnancy success rate, which it was suggested may have resulted from reduced fertilisation or implantation. In the second study, pregnant rats given 10 mg/kg bw/day of another commercially available extract (containing the equivalent of 0.2-0.4 % quercetin and kaempferol and 2-7 % ellagic acid), gave birth to male and female offspring which exhibited significant alterations in CYP activity, compared with untreated controls (Makaji *et al*., 2011).

131. The third study gave mice *ad libitum* access to water bottles throughout pregnancy, containing 1.78 or 2.66 mg/ml raspberry leaf extract (Hastings-Tolsma *et al*., 2022). Compared with untreated controls, both raspberry leaf groups exhibited significant reductions in litter size (viable and non-viable) and a non-statistically significant trend towards reduced neurodevelopmental behaviour, including increased time to righting, an increased geotaxis reflex time and reduced swim test scores. Compared with controls, the high-dose group also exhibited significant reductions in pup weight gain at postnatal days 4 and 5, based on the graphical data presented (although a comment in the text suggested pup weight was higher in the high-dose group compared with the control group at postnatal day 5). According to the authors, the changes in the high-dose group were accompanied by a non-statistically significant trend towards reduced gestation length (although a statistically

significant *p*-value of <0.04 was stated in the text with respect to this outcome).

132. Ethanolic raspberry leaf extracts also demonstrated an ability to inhibit recombinant human CYP enzymes *in vitro*, with IC_{50} constants of 44, 47 and 81 µg/ml towards CYP1A2, CYP2D6 and CYP3A4, respectively (Langhammer and Nilsen, 2014). This may suggest that raspberry leaf could have the potential to interact with other drugs during pregnancy. Additional concern has been expressed that polyphenolic compounds in raspberry leaf may compete for iron absorption, thus promoting anaemia in women taking it during pregnancy, although no studies investigating this were identified in the present review or in a recent systematic review (Holst *et al.*, 2011; Bowman *et al.*, 2021).

133. There is additional uncertainty surrounding the health risk posed by potential contaminants in raspberry leaf, due to lack of research. However, there is evidence that some raspberry leaf products may contain cadmium levels above the WHO-recommended limit (0.3 mg/kg), along with traces of other heavy metals (but within current UK legal limits for supplements) (Kowalska, 2021; Veatch-Blohm *et al.*, 2021). They may also contain high pesticide levels exceeding the current UK legal limits for herbal infusions, such as boscalid, and fungal contaminants, which may be of concern in immunocompromised individuals (Sadlo *et al*., 2015; Veatch-Blohm *et al*., 2021).

Conclusions

134. Over the centuries, raspberry leaf has been recommended for a range of medicinal purposes. It is most commonly used during pregnancy to stimulate and facilitate labour and to shorten its duration, as well as other uses, such as alleviation of morning sickness or prevention of miscarriage. It is usually consumed as tea or tablets but also as tinctures.

135. Overall, there is a great deal of uncertainty surrounding the safety of raspberry leaf consumption during pregnancy, largely due to limited data from *in vitro*, animal and human studies. No research appears to have been conducted to evaluate whether raspberry leaf might have genotoxic or carcinogenic effects and only 3 human studies have assessed its safety when consumed during pregnancy. One of these was a double-blind, placebo-controlled, randomised trial in which nulliparous pregnant women were given 2.4 g raspberry leaf extract daily from 32 weeks' gestation (consumed as tablets in 2 separate 1.2 g doses). The other 2 were observational and included limited information about the dose, timing or duration of raspberry leaf use. None of the human studies found raspberry leaf consumption during pregnancy to be associated with adverse effects in mother or child (although one study reported an increased risk of caesarean delivery). However, each were of short duration, included limited sample sizes and suffered from other methodological limitations, such as variation in participants' baseline characteristics.

136. Case reports suggest raspberry leaf consumption during pregnancy may be associated with adverse effects on maternal health. These include diarrhoea, hypoglycaemia in those with GDM requiring insulin, and acute liver injury. Other case studies of women taking raspberry leaf during pregnancy have reported adverse neonatal effects, including epilepsy, petechiae and ecchymoses, convulsions and cerebral palsy. However, limited information was available in these studies about the doses taken and it is uncertain whether the adverse effects described were related to raspberry leaf consumption or to other factors, such as the use of other herbal products described in several of them.

137. Concern has been expressed within the lay press and by some medics that raspberry leaf use during pregnancy may promote miscarriage or premature labour. However, according to peer-reviewed sources, there is considered limited evidence to support this, except for 1 study which found that aqueous raspberry leaf extracts were able to contract human uterine

muscle *in vitro* at doses equivalent to 0.06 g/ml dried leaf (although another study reported a relaxant effect on uterine muscle in post-partum women given an oral dose of 1.3 g or more dried raspberry leaf extract or 20 oz. 5 % raspberry leaf tea).

138. A range of potential adverse effects associated with raspberry leaf exposure have been identified during animal studies, depending on factors such as the dose, preparation, animal species and mode of administration. These range from reduced weight gain and API through to transgenerational effects, such as alterations in CYP activity, convulsions and death.

139. Aqueous raspberry leaf extracts were able to reduce the viability of Chang liver and HEK 293 cells *in vitro* at doses of 1-100 mg/ml and to promote apoptosis in Chang liver cells at doses of 25-100 mg/ml, compared with untreated controls. Ethanolic raspberry leaf extracts also demonstrated an ability to inhibit recombinant human CYP enzymes *in vitro,* with IC_{50} constants ranging from 44-81 µg/ml. This may suggest that raspberry leaf could have the potential to interact with other drugs during pregnancy. Additional concern has been expressed that polyphenolic compounds in raspberry leaf may compete for iron absorption, thus promoting anaemia in women taking it during pregnancy, although no studies investigating this were identified in the present review or in a recent systematic review.

140. The uncertainty surrounding the safety of raspberry leaf during pregnancy is compounded by the lack of understanding as to its biological effects and active components. These may vary in concentration between preparations according to factors such as area of cultivation, which could potentially result in differences in their toxicity.

141. There is additional uncertainty surrounding the health risk posed by potential contaminants in raspberry leaf, due to lack of research. However, some products may contain high levels of pesticides - in excess of current UK legal limits for herbal infusions, as well as relatively high levels of cadmium

and traces of fungal contaminants, which may be of concern in some individuals.

Questions on which the Views of the Committee are Sought

Members are asked to comment on:

a) The risk to maternal health associated with raspberry leaf consumption.
b) Whether it is possible to derive a point of departure to be used in the risk assessment of raspberry leaf, based on the information presented in this discussion paper.
c) Any other points relating to this paper.

Secretariat

Sept 2022

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List of Abbreviations

ADI	Acceptable daily intake
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AMMC	3-[2-(N,N-diethyl-N-methylammonium)ethyl]-7-methoxy-4- methylcoumarin

API	Adiposity percentage
AST	Aspartate transaminase
BFC	7-benzyloxy-4-(trifluoromethyl)-coumarin
BMI	Body mass index
BQ	7-benzyloxyquinoline
bw	Body weight
CEC	3-cyano-7- ethoxycoumarin
CI	Confidence interval
CYP	Cytochrome P450
DES	Diethylbestrol
DMSO	Dimethylsulfoxide
EC_{50}	Half-maximal effective concentration
EFC	7-ethoxy-4-trifloromethyl-coumarin
EMA	European Medicines Agency
ERES	Ethoxyresorufin
FSA	Food Standards Agency
GDM	Gestational diabetes mellitus
HBGV	Health-based guidance value
HPLC	High-performance liquid chromatography
IC_{50}	Half-maximal inhibitory concentration
LCMS/MS	Liquid chromatography mass spectrometry/mass spectrometry
LOQ	Limit of quantification
MFC	7-methoxy-4-(trifuoromethyl)-coumarin
MHRA	Medicines and Healthcare Products Regulatory Agency
MRES	Methoxyresorufin
MRL	Maximum residue limit
NDNS	National Diet and Nutrition Survey

NP	Non-pregnant
RLE	Raspberry leaf extract
RLE-H	High-temperature, high-pressure raspberry leaf extract
RLP	Raspberry leaf powder
SACN	Scientific Advisory Committee on Nutrition
TG2	Transglutaminase 2
TUL	Tolerable upper intake level
UKTIS	UK Teratology Information Service
WHO	World Health Organisation

Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment

Effects of Raspberry Leaf on the Maternal Diet

Search Methodology

1. The following electronic databases were searched for relevant articles published from inception to Apr 2022: LitFetch (which includes material from PubMed, Scopus, Ebsco (Food Science Source) and Springer); ScienceDirect and Cochrane Library. Google and Google Scholar were also searched. The searches were conducted on various dates between 1st Apr 2022 and 22nd Apr 2022.

2. The search terms used included 'raspberry leaf' OR '*Rubus idaeus*' AND: ('safety' OR 'tox*' OR 'pregnan*' OR 'maternal' OR 'exposure' OR 'consumption' OR 'indications' OR 'uses' OR 'childbirth' OR 'transgenerational' OR 'transgenic' OR 'convulsion' OR 'uterine contract*' OR 'development' OR 'interactions' OR 'gestation' OR 'caesarean' or 'contaminant' OR 'heavy metal' OR 'mycotoxin' OR 'pesticide' OR 'residue').

3. The references from extracted papers were searched for citations not captured in the literature search. Only articles published in English were included, due to the linguistic abilities of the reviewer.

4. The UKTIS was also asked for information on any enquiries relating to maternal raspberry leaf use and any reports of adverse effects in pregnant women or their newborn infants received since its inception in 1983 to Apr 2022. This included information relating to the type, dosage, duration and timing of raspberry leaf taken and any pregnancy outcomes captured through follow-up.

Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment

Effects of Raspberry Leaf on the Maternal Diet

Online Dose Recommendations for Raspberry Leaf During Pregnancy

Source	Dose			Preparation
	Tea/Extract	Tablets	Tincture	
(Jen Jester, no date)	Up to four cups daily.	N/a	N/a	N/a
(Edwards *et al.*, 2015)	4-8 g as an infusion daily.	N/a	4-8 ml (1:1 in 25 % alcohol) three times daily.	N/a
(*Raspberry leaf tea - yes or no?* | *Mumsnet*, no date)	1-3 cups daily as tea after 36-37 weeks of gestation.	N/a	N/a	N/a
(Gerard, 1597, cited in McFarlin *et al.*, 1999)	N/a	N/a	N/a	Tea: 2 g steeped in 240 ml boiling water.
(Kothari, no date)	One cup daily at 32 weeks, increasing to 2-3 cups per day by 37 weeks.	N/a	N/a	N/a
(*Raspberry Leaf Tea Benefits for Pregnancy - Does It Really Work???*, 2019)	One cup daily, increasing to up to three cups per day in the third trimester, or at approx. 35 weeks.	N/a	N/a	N/a
(*How to Harvest and Preserve Red Raspberry Leaf Tea*, 2015)	N/a	N/a	N/a	Tea: 1 tsp per 8-ounce cup of boiling water.
(Bonet, 2021)	1-3 cups daily.	N/a	N/a	N/a
(*maternal — Blog*, no date)	N/a	N/a	N/a	Tea: ‘1-2 teaspoons of dried leaf per cup of hot

				water' OR '4 tablespoons dried herb in a quart of hot water' OR '4 [tbsp] of the dried leaf in a quart of cold water.'
('"I encourage mothers to use raspberry tea throughout the pregnancy and more heavily in third trimester', no date)	Leaf hot infusion: 2-3 cups daily or more (as desired). Leaf cold infusion: can be drunk in same dosages as hot infusion.	4-8 g, 1-3 times daily.	2-4 ml three times daily OR 4-8 ml (3/4-1 tsp) three times per day.	Leaf hot infusion: 2tsp of dried leaf added to a cup of boiling water. Leaf cold infusion: 2 tsp in one cup of cold water. Leaf tincture: 1:5 with 35%-50% alcohol.
(Parsons, 1999, cited in Palmer, 2018)	Teabag cups: one cup daily during the first trimester; two cups daily during the second trimester; up to 4-5 cups during the last trimester. Loose leaf tea: 2-3 cups per day, especially after 28 weeks of pregnancy.	Two 300/400 mg tablets with each meal (three times daily) from 32 weeks.	Dose dependent on tincture strength.	Loose leaf tea: add 1 tsp of herb to a cup of boiling water, stir, sit for 10 minutes, strain and sip.
(Australia, 2021)	1-2 cups daily	N/a	N/a	N/a
(*Raspberry leaf tea? Please share your experiences!*, no date)	One cup of tea daily at 32 weeks, increasing every few days by one cup until taking 3-4 cups daily.	One tablet daily at 32 weeks, increasing every few days by one tablet until	N/a	N/a

		taking 3-4 tablets daily.		
(*The Benefits of Raspberry Leaf Tea During Pregnancy*, no date)	One cup of tea per day from 27 weeks of gestation, increasing to 2-4 cups per day by week 35.	N/a	N/a	N/a
(Expectancy, 2022)	One cup daily at approx. 32 weeks, increasing gradually every few days to 3-4 cups.	One tablet (typically available as 300-400 mg doses) daily at approx. 32 weeks, increasing gradually every few days to 3-4 tablets.	N/a	N/a
('Red Raspberry Leaf Tea - Benefits For Pregnancy and Labor | BellyBelly', 2011)	At least 2-3 cups (max 4-5) daily during the third trimester but one cup per day during the first trimester is 'fine.'	Two 300-400 mg tablets with each meal three times a day from 32 weeks.	N/a	Tea: add 1 tsp of raspberry leaf to a cup of boiling water.
(Health, no date)	One cup daily or if not experiencing any uterine sensations, increase to two cups per day (provided at due date or the 37th week).	N/a	N/a	Tea: steep 1-2 tsp raspberry leaf in a cup of boiling water.
(Ellis, no date)	One cup daily, gradually increasing to 4-5 cups a day.	Two tablets, three times daily from 32 weeks.	N/a	N/a
(*The best raspberry leaf teas for pregnancy 2022*, no date)	One cup daily. May increase gradually at 34 weeks to 2-3 cups.	N/a	N/a	N/a

('Red Raspberry Leaf Pregnancy Tea – A Nourishing Tonic For Two', 2019)	One cup daily at 32 weeks, gradually increasing to three cups as the due date approaches.	N/a	N/a	N/a
(Hearth and Home Midwifery, 2022)	1-2 cups daily throughout the third trimester.	N/a	N/a	Tea: steep two handfuls of the dried herb in '1 qt cold water.'
(ASKINGLOT, 2022)	One cup daily from 32 weeks, gradually increasing to three cups.	N/a	N/a	N/a
(*Red Raspberry Leaf Tea During Pregnancy Tones Your Uterus*, no date)	One cup daily during the first trimester, two cups daily in the second trimester and three cups daily during the third trimester.	N/a	N/a	Tea: add 2 tsp of dried tea into a strainer and pour one cup of boiling water over the top.
(Darby, no date)	1-3 cups daily starting in the second trimester.	N/a	N/a	N/a
('Raspberry Herbal Tea and Pregnancy', 2019)	Three cups daily during the last 3-4 weeks of pregnancy.	N/a	N/a	N/a
(J Jester, no date)	1-2 cups daily in the last 6-8 weeks of pregnancy. 'Drink plenty during labor…drink postpartum to cleanse the uterus and encourage healthy breastmilk production.'	N/a	N/a	N/a
(*8 Ways to Start Labor Naturally*, 2018)	1-2 cups daily from 32 weeks of gestation.	N/a	N/a	N/a
(*Red Raspberry Leaf Tea - Precautions & Side Effects - Teatoxlife*, no date)	1-2 cups daily, gradually increasing to three cups per day.	N/a	N/a	N/a
(*Red Raspberry Leaf Tea: Pregnancy,*	1-3 cups daily.	N/a	N/a	N/a

Benefits and Side Effects, 2018)				
(Medical News Today, 2021)	1-3 cups daily.	N/a	N/a	Tea: add 1 tsp crushed or dried raspberry leaves to a cup and pour over boiling water.
(*Brewing a Baby? How Red Raspberry Leaf Tea Benefits Your Pregnancy and More*, 2022)	'1-3 cups a day is safe…if…concerned about preterm labor…drink in the third trimester.'	N/a	N/a	N/a
(*RASPBERRY LEAF - Drugs in Pregnancy and Lactation: Tenth Edition*, no date)	'Typical doses…are 1.5–2.4 g/day.'	N/a	N/a	N/a
('Red Raspberry Leaf Tea for Pregnancy: All You Need to Know', 2021)	One cup daily from as early as 14 weeks of gestation. Increase to two cups daily during the third trimester until due date.	N/a	N/a	Tea: add 1 tbsp of loose red raspberry leaf tea to every cup of boiling water.
(*Red Raspberry - Cancer Care of Western New York*, no date)	2-3 cups daily.	N/a	N/a	Tea: add one cup of boiling water to 1-2 tsp dried leaf.
(*Red Raspberry Leaf Tea - Benefits, Pregnancy, Labor, and Side Effects*, no date)	One cup daily from 32 weeks of gestation, gradually increasing to 2-3 cups daily until delivery.	N/a	N/a	N/a
(Mills and Bone, 2004)	12-24 g/day of dried leaf or by infusion. 12-24 ml/day of a 1:1 liquid extract. 4.5-1.4 ml/day of a 1:2 liquid extract or equivalent in tablet or capsule form.	N/a	N/a	N/a