Toxicity

In this guide

8. EAs modulate the function of noradrenaline, dopamine and serotonin neurotransmitters. The structural similarities of EAs to these neurotransmitters allow them to activate or block the neurotransmitter receptors and modify neurotransmitter release and reuptake. EAs produce peripheral effects such as

uterine contractions and vasoconstriction, and central nervous system (CNS) effects such as induction of hypothermia and emesis. (Arroyo-Manzanares et al., 2017; Cassady et al., 1974; EFSA, 2012; Fitzgerald and Dinan, 2008; Schardl et al., 2006).

Toxicokinetics

9. In both humans and experimental animals EAs are incompletely absorbed from the gastrointestinal (GI) tract, the intestinal absorption of hydrogenated ergot peptide alkaloids generally varying between 10 and 30 %. EAs are subjected to oxidative biotransformation, involving cytochrome P450 3A4 (CYP3A4), via hydroxylation in the liver. However, while EAs are metabolised by CYP3A4 they can also inhibit its activity (Aellig et al., 1977; Eckert et al., 1978; Little et al., 1982; Olver et al., 1980; Wyss et al., 1991).

10. Studies have shown that P450 monooxygenase and its clusters play a key role in the biosynthesis of many EAs, with clavine oxidase (CloA) playing a role in EAs that are derivatives of LA (Gerhards et al., 2014, Haarmann et al., 2006; Mulac and Humpf, 2011; Young et al., 2015).

11. No studies were available on the toxicokinetics of dietary EAs in humans. However, human data were available on ergotamine used as a pharmaceutical to treat Parkinson’s disease. Absorption of ergotamine from the GI tract was poor after oral/sublingual administration and bioavailability was further reduced by high pre-systemic hepatic metabolism. Ergotamine tartrate can also be given rectally to improve absorption, yet bioavailability is still ≤ 5 %. Caffeine is sometimes included in oral and rectal preparations in an effort to improve absorption; the effectiveness of this is, however, still unclear (Silberstein and McCrory, 2003; Tfelt-Hansen et al., 2000).

Acute Toxicity

12. Species differ in their sensitivity to EAs, rabbits being the most sensitive species with lethal dose (LD)50 values between 0.9 and 3.2 mg/kg body weight (bw) following intravenous (i.v.) injection. The LD50s were determined for a series of naturally occurring and (semi-) synthetic EAs by i.v., subcutaneous (s.c.) and oral exposures (in 2 % gelatine) in mouse, rat and rabbit (Griffith et al., 1978). All naturally occurring EAs demonstrated a low oral acute toxicity compared to i.v. administration, indicating low absorption and high pre-systemic metabolism via the oral route. Based on the oral LD50s (27.8 – 1200 mg/kg), EFSA

concluded that, overall, EAs exhibit moderate oral acute toxicity (EFSA, 2012).

13. In repeat oral dose studies in rats there were no significant differences in the toxicity of ergotamine, ergometrine and α-ergocryptine, with no-observed-adverse effect levels (NOAELs) ranging from 0.22 - 0.60 mg/kg bw per day (EFSA, 2012).

14. Exposure to cereal grains contaminated with EAs can lead to a condition called ergotism (Guggisberg, 2003). There are two main types of ergotism, gangrenous and convulsive. The gangrenous form is caused by the strong vasoconstrictive properties of some EAs, which result in restriction of blood flow to peripheral parts of the body (ischemia). In the convulsive form, tingling is followed by neurotoxic symptoms such as hallucinations, delirium, and epileptic-type seizures. It has been suggested that as well as a high concentration of EAs, a deficiency in vitamin A could be a causative factor inducing convulsive ergotism. Additional symptoms of ergotism are lethargy and depression (Arroyo-Manzanares et al., 2017; EFSA, 2012).

15. Because EAs act on several neurotransmitter receptors, particularly adrenoceptors, dopamine and serotonin receptors, EFSA considered neurotoxicity to be their main acute effect, with symptoms such as restlessness, miosis or mydriasis (contraction and dilation of the pupils), muscular weakness, tremor and rigidity in mammals. In humans, acute effects are directly related to receptor antagonism and include diarrhoea, and loss of consciousness.

Chronic toxicity

16. A study by Valente et al. (2020) reported a decrease in serum 5-hydroxytryptamine (5-HT) levels in bovine species fed dietary concentrations of egrovaline for 15 days (0, 0.862, 1.282 mg/kg dry diet ad libitum) or dosed directly into the rumen with ergovaline for 7 days (15 µg/kg bw).

17. A study by Korn et al. (2014) reported spontaneous alopecia, erosions, crusts and necrosis, specifically of the tail area, which occurred exclusively in young rabbits aged 113 ± 20 days (14 out of 103 rabbits) from a colony fed with hay and a commercial pelleted feed. The results of the study indicated that EAs may have been the cause of the tail necrosis, with immunoassays on blood samples showing a mean and maximum EAs concentration of 410 µg/kg and 1,700 µg/kg, respectively. In addition, EAs were detected in the faeces of the affected rabbits at levels up to 200 µg/kg. The mean and maximum dietary intakes of total EAs were 17 and 71 µg/kg bw, respectively. Other toxins, such as

fusarium toxin, were also detected in the feed, but at levels which, according to the authors, did not explain the observed effects.

18. Repeated dosing with various EAs, resulted in ischaemia, particularly in the extremities (e.g., tails) of rats, decreased body weight gain and changes in the levels of some hormones such as follicle-stimulating hormone (FSH), thyroid-stimulating hormone (TSH) and luteinizing hormone (LH) (Janssen et al. 2000; JECFA, 2023). Tail gangrene was observed in rats 5 - 7 days after a single i.p. exposure to 25 mg/kg bw ergotoxine (a mixture including ergocornine, α- and β-ergocryptine, and ergocristine) (Griffith et al., 1978). The NOAELs were 0.22 - 0.60 mg/kg bw per day. No major quantitative difference in the toxicity of ergotamine, ergometrine and α-ergocryptine was observed (EFSA, 2012; JECFA, 2023).

19. No data were available on the chronic toxicity of EAs from dietary exposure in humans. However, limited information was available from the use of ergot containing medications. Case studies of long-term use of EA medication (doses from 1 to 2 mg ergotamine titrate) for migraine headaches reported severe lower extremity claudication (pain in the limbs) due to chronic arterial insufficiency (Bogun et al., 2011; Fröhlich et al., 2010; Garcia et al., 2000; Silberstein and McCrory, 2003). In all instances treatment was discontinued and patients were also asked to discontinue the use of caffeine and cigarettes. Anti-platelet therapy was used to successfully reverse the symptoms.

20. To minimize toxicity and avoid adverse effects such as nausea, vomiting, weakness, muscle pains, paraesthesiae and coldness of the extremities from acute migraine treatment with ergotamine, it was recommended that the maximum dose should not exceed 10 mg per week. (Orton and Richardson, 1982; Perrin et al., 1985).

21. Bromocriptine is a synthetic compound with an affinity to dopamine receptors due to its structural similarities to a variety of EAs. It is therefore used as a treatment for Parkinson’s disease and to supress prolactin levels. However, several case reports, involving a total of 510 patients, showed adverse side effects in 34 % of patients with Parkinson’s disease receiving high doses of bromocriptine, 31-100 mg/day (Bernard et al., 2015). A number of severe adverse events were also reported in patients receiving bromocriptine for suppression of lactation (Lieberman and Goldstein, 1985).

Genotoxicity and Carcinogenicity

22. No genotoxicity effects were demonstrated for ergotamine tartrate (Et) in a *Salmonella typhimurium (St)* assay (10-10,000 µg/plate for 48 hours) and mouse lymphoma TK+/- assay (7.7-108 µg/mL for 4 hours) (Seifried et al., 2006). Roberts and Rand (1977) reported that ergotamine induced chromosomal abnormalities in human lymphocytes and leukocytes *in vitro*. In a study by Dighe and Vaidya (1988) ergotamine, ergonovine and methylergonovine induced sister chromatid exchange (SCE) frequencies *in vitro* in cultured Chinese hamster ovary (CHO) cells, while ergocristine and α-ergocryptine showed a weak and no effect, respectively.

23. Due to limited and contradictory data on the genotoxic and mutagenic effects of EAs, EFSA considered the available genotoxicity studies to be insufficient, except for ergotamine, concluding that the available data on ergotamine did not indicate bacterial or mammalian cell mutation (EFSA, 2012). Taking into account all the available information, the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2023) concluded that naturally occurring EAs do not raise concerns for genotoxicity.

24. Bromocriptine has been shown to cause an increase in uterine tumours in rats, due to its inhibition of prolactin secretion, with a lifetime of relative hyperprolactinemia. However, as the mechanism proposed in the rat is the reduced luteotrophic effect of prolactin, and due to the distinctly different mechanisms involved in female reproductive hormonal regulation between humans and rat, the mode of action for these tumours in the rat is generally considered to have no relevance to humans (Harleman et al., 2012).

25. EAs are not considered to pose a carcinogenic risk to humans (EFSA, 2012; JECFA, 2023) but they have not been assessed by the International Agency for Research on Cancer (IARC). However, they have been suggested to be possible cytostatic agents, with a possible role as anti-cancer agents (De Ruyck et al., 2015). Experiments in rodents showed that ergotamine, ergocryptine and ergocornine were able to suppress the growth of pituitary tumours *in vivo* (MacLeod and Lehmeyer, 1973)

26. More recently a range of mRNA microarray studies investigating the cytotoxic activity of EAs on a range of human cancer cell types reported strong inhibitory effects for 1-propylagroclavine and dihydroergocristine against genes associated with the progression of leukaemia. The cytotoxicity pathway is not yet fully understood, but preliminary results suggested that EAs have the potential to be used for the treatment of otherwise drug-resistant and refractory tumours via the inhibition of prolactin release from the anterior pituitary gland (Cassady et al.,

1974; Mrusek et at., 2015).

Reproductive and Developmental toxicity

Reproductive effects in animals

27. EAs have a number of well-established effects on the reproductive process in rodents, including prevention of pregnancy, predominantly due to interference with implantation, and embryotoxicity. These adverse effects in rodents have generally been observed at higher doses than the lowest-observed-adverse-effect levels (LOAELs) in the repeat dose studies (EFSA, 2012).

28. Effects on FSH levels were observed in rats fed a 0, 4, 20, 100 or 500 mg ergocryptine/kg diet for 28 - 32 days. In females there was trend for FSH to decrease with dose, but there was appreciable variation and this was not statistically significant, while in males there was no consistent effect. There was a dose-dependent increase in LH in males but not in females. Prolactin levels were significantly decreased in both males and females at dose above 4 mg/kg bw per day. There was a decrease in thyroxine (T4) levels in both sexes, with males more sensitive than females (Janssen et al., 2000).

29. Cows that were exposed to EAs via tall fescue grazing had a 41 % lower conception rate than those grazing an EA-free pasture. The period of concern for EA negatively affecting conception was identified as the time between ovulation and the first six days of embryonic development (Klotz et al., 2019). EAs were also shown to directly alter bovine sperm motility and morphology, indicating that EAs may hinder cattle reproductive rates (Page et al., 2019).

30. Three yearling colts were fed a diet rich in EAs for 80 days. Four colts were fed a control diet. In spermatocytes from colts fed EAs, there was a significant, deleterious effect in the establishment and/or maintenance of partial synapsis of the sex chromosomes. The authors suggested that exposure of colts, prior to maturity, could impair or alter normal sexual maturation, which lead to fertility issues when these colts became sexually mature. There was little or no effect in mature stallions. (Fayrer-Hosken et al., 2012; 2013).

31. Studies in livestock also reported reduced reproductive performance, particularly in female cattle, after EAs exposure. Regional vasoconstriction and corresponding decreased blood flow to reproductive tissues was observed, along with a decreased dry matter intake, and/or increased body temperature, leading the authors to conclude that the effect of EAs was both direct and indirect (Poole

and Poole, 2019).

32. Limited information was available on the effects of EAs exposure during pregnancy itself, in particular the effects on the vascular system supporting the growing fetus. A study by Duckett et al. (2014) examined fetal growth in sheep following maternal exposure to EAs at a concentration of 0.8 µg/g diet of ergovaline during gestation, this is the equivalent to 0.011 µg/kg bw per day based on a body weight of 70 kg. Exposure to EAs during mid and/or late gestation in ewes reduced fetal growth. A more recent study in ewes indicated that maternal blood supply to the placenta appeared to be resistant from adverse effects of EAs, but umbilical vasculature was not, which could adversely influence normal fetal growth (Klotz et al., 2019). *In utero* exposure to EAs in pregnant ewes, especially during phase two of gestation altered fetal growth, muscle fibre formation, and micro ribonucleic acid (miRNA) expression (Greene et al., 2019). Ergovaline was a potent vasoconstrictor in the bovine umbilical and uterine arteries and reduces blood flow to developing placental tissues and fetuses (Klotz et al., 2015). Placental weight reduction was highly correlated with fetal birthweight and high exposure to EAs in ruminants can result in additional adverse effects such as hyperexcitability, hypermetria, and tremors (Britt et al., 2019; Klotz et al., 2015).

Pregnancy in humans

33. Data from trials on the use of EAs (ergometrine and methylergometrine) as uterotonic medication suggested that EAs may decrease mean blood loss from both mother and child by at least 500 mL and increase maternal haemoglobin levels in the blood. However, the results also suggested the treatment increased the incidence of adverse effects such as increased blood pressure and pain after birth (Liabsuetrakul et al., 2018).

34. A review of the Hungarian Case-Control Surveillance of Congenital Anomalies database, 1980-1986, suggested a possible link between the use of purified ergotamine and neural tube defects in humans. Ergotamine was used to treat acute migraine and a mean daily dose of 1.5 mg ergotamine during the 2nd month of pregnancy was associated with a higher risk for neural-tube defects. This was based only on three cases (Ács et al. 2006; Czeizel, 1989). There were two case reports of a possible association between the use of ergotamine during early pregnancy and the development of Möbius sequence in children (Smets et al., 2004; Graf and Shepard, 1997). Möbius sequence is a rare congenital disorder defined by the paralysis of the 6th and 7th cranial nerves in combination with

various odontological, craniofacial, ophthalmological and orthopaedic conditions (Kjeldgaard Pedersen et al., 2017). Vascular disruption has been suggested as one possible explanation for the pathogenesis of Möbius sequence. However, Ács et al. (2006) found no evidence for an association between the use of ergotamine during pregnancy and Möbius sequence. Ergotamine has also been reported to cause vasospasm and a prolonged and marked increase in uterine tone (Smets et al., 2004; Graf and Shepard, 1997).

35. A single case report of a 36-year-old patient suggested that exposure to methylergonovine maleate (an EA derivate) at a critical stage of organogenesis was a possible cause of the development of sirenomelia. Sirenomelia is a rare and deadly condition characterized by fusion of the lower limbs, lower spinal column defects, severe malformations of the urogenital and lower GI tract, and an aberrant abdominal umbilical artery (Cozzolino et al., 2016).

Lactation in animals

36. Prolactin has important biological functions, including but not limited to lactation, reproduction, and metabolism and the inhibition of prolactin production by EAs has been seen in humans, laboratory animals, and livestock animals (Arroyo-Manzanares et al., 2017; Prendiville et al., 2000). Intraperitoneal injection of 1 mg ergocornine methane sulfonate in lactating rats temporarily inhibited milk production, the effect being prevented by treatment with prolactin (Zeilmakla and Carlsen, 1962). The potency of EAs to inhibit prolactin secretion in rats decreased in the following order: ergocornine > ergocryptine > dihydro-ergocryptine > dihydro-ergocornine > ergotamine > ergometrine (Griffith et al., 1978). Shaar and Clemens (1972) suggested that EAs directly affect the pituitary therefore preventing prolactin secretion, resulting in partial inhibition of lactation. Decreases in prolactin levels were observed in rats of both sexes with increasing amount of α-ergocryptine in the diet. In males, T4 was also significantly decreased and accompanied by a decrease in free thyroxine (FT4).

Lactation in humans

37. In a review of a randomised clinical trial to evaluate the effects of EAs on milk secretion postpartum, 30 women received an injection of 0.2 mg methylergobasine immediately after delivery followed by three tablets of 1 mg of ergotamine tartrate given daily (orally) for six days post-partum. The treatment had no significant effect on either the weight of the infant or the quantity of milk consumed (Jolivet et al., 1978). A study by Arroyo-Manzanares et al (2017)

addressed the similarities of the actions of EAs to those of monoamine neurotransmitters and provided evidence that EAs have the ability to act on the secretion of adrenocorticotropic hormone (ACTH), prolactin (PRL), LH and FSH.

Immune effects

38. Limited to no information was available on the effects of EA on the immune system. EFSA did not consider immunotoxicity in their assessment in 2012, while JECFA noted only that a high alkaloid content in the diet fed to rabbits for four weeks adversely affected the immune system, however no further information was available and potential immunotoxicity was not further discussed (JECFA, 2023).

39. A literature review was undertaken to establish whether any new information was available since the 2023 JECFA assessment, however no scientifically relevant information/data could be identified regarding the immunotoxicity of EAs.