Toxicology

In this guide

This is a paper for discussion. This does not represent the views of the Committee and should not be cited.

9. EFSA carried out a full assessment of CIT in 2012 and this has been used as a baseline point for the following hazard characterisation. In addition, a literature search was carried out from 2012-2024, ensuring relevant data published after the EFSA assessment were also considered here. The key studies used in the RIVM risk assessment (2015) were retrieved as part of this literature search. The following sections therefore cover, in brief, all relevant toxicological information up until 2024.

Toxicokinetics

10. The available information on CIT showed it is eliminated predominantly by renal excretion; approximately 75 % of radiolabelled citrinin (14C-citrinin) given to pregnant rats by subcutaneous administration was recovered in urine (Reddy et al., 1982a). A study by Meerpoel et al. (2020b) demonstrated differences in the toxicokinetic properties of CIT between pigs and chickens, including clearance being much slower in pigs than in chickens (Meerpoel et al.,

2020b).

11. An *in vitro* study in Chinese hamster lung fibroblast cells demonstrated that the toxic potency of the metabolite DH-CIT was less than CIT (Föllmann et al., 2014) while the interaction of DH-CIT with albumin from different species *in vitro* did not show significant difference between species (Faisal et al., 2019).

12. A study in human volunteers demonstrated that ingested CIT undergoes conversion to dihydrocitrinone (DH-CIT) which is then excreted in the urine along with the remaining parent compound (Degen et al., 2018).

13. A study by Singh (2012) suggested that CIT can cross the placenta (discussed further in the developmental section, see paragraph 31).

Experimental toxicity

Genotoxicity

14. EFSA concluded that the available data indicated that CIT is not mutagenic in conventional bacterial assays either with or without metabolic activation by S9 fraction (EFSA, 2012). Mutagenicity in the Ames test was reported in only one study when rat hepatocytes were used as the activating system (Sabater-Vilar et al., 1999). In mammalian cells *in vitro*, CIT did not induce DNA single-strand breaks, oxidative DNA damage or sister chromatid exchanges (SCE) but induced micronuclei, aneuploidy and chromosomal aberrations.

15. In vitro assays published since the EFSA opinion showed that CIT induced a dose dependent increase in micronuclei (MN) frequencies, chromosomal aberrations and sister chromatid exchanges (Anninou, 2014; Föllmann, 2014; Tsai, 2023).Tsai et al. (2023) concluded that CIT exposure activated cancer and cell cycle-related signalling pathways when human embryonic kidney 293 (HEK293) cells were treated for 3 and 30 days (Tsai, 2023).

16. *In vivo*, CIT induced chromosome abnormalities and hypodiploidy in the bone marrow of mice exposed at concentrations of 5-20 mg/kg bw for eight weeks, by oral administration (Jeswal, 1996).

Carcinogenicity

17. F344 rats were fed diet containing CIT in the diet at 0.1 % (estimated to be 0.1 mg/kg bw/day) for 80 weeks; the kidney was identified as the main target organ with reported induction of adenomas (Arai and Hibino, 1983). The first renal tumour was seen at necropsy week 52. Renal adenomas were seen in 35 of the 48 rats that had survived after 40 weeks.

18. A series *of in vivo* studies by Kuroda (2013) administered CIT to rats by gavage at 20-40 mg/kg bw for a maximum of 28 days. The maximum dose of 40 mg/kg was decreased to 30 mg/kg from day four due to decreases in body weight. Regenerative tubules were observed in the kidney cortex in the high dose group, while labelling index of proliferating cell nuclear antigen (PCNA)- positive cells was significantly increased at both doses. The authors suggested that the increase in cyclin encoding genes (*Ccna2*, *Ccnb1*, Ccne1) and its transcription factor (E2f1) indicated induction of cell cycle progression at all tested doses. Cyclin B1 specifically is ubiquitously expressed in humans and plays a key role in controlling the cell cycle transitions.

Nephrotoxicity

19. In vitro, the acute cytotoxic effects of both DH-CIT and CIT were significantly decreased on a Madin-Darby canine kidney (MDCK) cell line, in the presence of albumin (Faisal et al., 2019).

20. In vivo, the acute lethal dose of CIT ranged from 19-134 mg/kg bw depending on species and route of administration (EFSA, 2012). The main changes in pathology following CIT administration were degeneration and necrosis of the kidneys in all species indicating nephrotoxicity. Repeat dose studies assessed by EFSA (2012) confirmed the nephrotoxicity of CIT and highlighted the differences in susceptibility between species, showing guinea pigs and dogs were more sensitive than hamsters. Necropsy showed histopathological changes in the kidneys of all species tested (except hamsters), which were consistent with the acute signs observed.

21. In a study by Lee et al. (2010), CIT was given in the form of fermented RMR containing different concentrations of CIT (1, 2, 10, 20 and 200 mg/kg). At the highest dose tested (stated by EFSA to be equivalent to 20 µg CIT/kg bw per day) no toxicologically significant alterations in body weight gain, daily feed intake, organ weight and serum biochemistry as well as histopathology of livers and kidneys were observed.

22. A repeat dose study by Jagdale et al. (2020) which treated rats daily by gavage with 25 µg/kg bw or 100 µg/kg bw CIT/day for 28 days reported adverse histopathological changes in the kidney and the spleen at the higher dose. No significant histological changes were reported in animals dosed with 25 µg/kg bw.

23. A 60-day study in rabbits suggested that at low concentrations, CIT (15 mg/kg feed estimated to be 0.45 mg/kg bw/day) induced apoptosis in a time dependent manner and lipid peroxidation in the rabbit kidney, which according to the authors, appeared to play a major role in the pathogenesis of nephrotoxicity (Kumar et al. 2014).

Immunotoxicity and Immunomodulation

24. EFSA concluded in 2012 that the data on immunotoxicity of CIT were incomplete and often non-specific and therefore did not allow for a conclusive evaluation.

25. Since the EFSA opinion, *in vitro* mammalian cell assays reported to show evidence of immunomodulatory and immunotoxic effects of CIT (Sugiyama et al., 2013: abstract only; Islam et al., 2012; Xu et al., 2022) have been published.

26. In vivo, mice treated orally with CIT (1, 5, or 10 mg/kg bw) showed reduced levels of serum immunoglobulin M (IgM) in a dose dependent manner, but no significant changes in immunoglobulin A (IgA),immunoglobulin E (IgE) and immunoglobulin G (IgG). Changes in the regulation of the different immune cell populations were reported in the spleen, mesenteric lymph nodes and small intestine at 1 mg/kg bw. (Islam et al., 2012).

Developmental and reproductive toxicity

27. EFSA considered CIT to show reproductive toxicity with teratogenic and embryotoxic effects based on data from *in vitro* and *in vivo* studies (EFSA, 2012). However, *in vivo* studies also reported maternal toxicity at the same dose, including nephrotoxicity, indicating that the reproductive, teratogenic and embryotoxic effects of CIT may be secondary to maternal toxicity.

28. Kinetic investigations in pregnant rats provided no conclusive data about the percentage of CIT that crosses the placenta (Reddy et al., 1982b). Therefore, EFSA could not determine the extent to which the offspring were exposed based on the available data.

29. Since the 2012 EFSA opinion, limited data has been published on the reproductive and developmental effects caused by CIT. However, as the assessment focusses on maternal diet and maternal outcomes, it should be noted that the doses at which these effects were observed in the published studies were in exceedance of EFSA's level of no concern for nephrotoxicity.

30. A repeated oral dose toxicity study by Hayashi et al. (2012) exposed female mice to 0, 1.25 or 7.5 ppm CIT for 70 days in drinking water. No effects on body weight, food consumption or clinical signs were observed and except for a slight increase in relative ovary weight, no other effects on kidney, liver or ovary were observed. A second experiment was carried out exposing female mice to 0, 15 or 30 ppm CIT for 90 days in drinking water. A significant decrease in relative liver weight and decreases in water consumption were observed in the lower (15 ppm) dose group, while decreases in body weight were observed in the higher treatment group (30 ppm). Both absolute and relative ovary weights increased accompanied by large follicles at ≥ 15 ppm (the authors estimated this was equivalent to 2.25 mg/kg body weight/day).

31. Singh et al. (2012) administered CIT (10 mg/kg feed estimated to be 1 mg/kg bw/day) to pregnant rats from gestational day (GD) 6-20, showing a significant increase in the percentage of apoptotic cells in kidneys of dams and foetuses. The effects caused by CIT administration on dams and foetuses were not reported, but toxicity as a result of apoptotic cells in the kidneys was inferred by the authors.

32. In a later one generation study by the same authors (Singh et al. ,2014) male and female rats were administered 1, 3 and 5 mg/kg CIT in feed (estimated to be 0.1, 0.3 and 0.5 mg/kg bw/day)
during mating and during organogenesis. Clinical signs observed included increased water intake, dullness, rough hair coat and polyuria. Mortality was not observed in the dams. Reproductive effects included reduced foetal bodyweight, reduced crown-rump length and increased number of malformations.

33. Newly fertilised zebrafish eggs were exposed to concentrations of 0.78-50 µM CIT before individuals reached free-feeding stage, i.e. while the zebrafish are still embryos prior to reaching the juvenile stage of development. (Csenki et al., 2021). Results showed no mortalities but exposure to 50 µM CIT led to pericardial oedema, blood accumulation, incorrect heart looping, and reduced the size of cardiac chambers.

Epidemiological studies

34. EFSA evaluated two studies in humans (Hetherington, 1941; Ambrose, 1946), however both focussed on inhalation of citrinin powder. EFSA drew no conclusion from these studies, and the effects are not considered to be relevant to the maternal diet.

35. The literature search undertaken here retrieved epidemiological studies specifically on pregnant women following citrinin exposure. However, no studies specific to the UK were available.

36. CIT and DH-CIT have been reported in urine from different human cohorts from Belgium, Czech Republic, Portugal, Germany, Haiti, Bangladesh, Nigeria, Turkey, and Tunisia (Narváez et al., 2021). CIT has also been detected in the breast milk and urine of mothers and the urine of exclusively breastfed infants in two Nigerian communities (Ezekiel et al., 2022).

37. Three biomonitoring studies were carried out to measure the concentration of CIT and DH-CIT in pregnant women, infants and children in Bangladesh (Ali and Degen, 2020; Kyei et al., 2023, 2022). CIT was detected in 61 % of the urine samples collected from pregnant women and dietary exposure to CIT, based on urinary levels, was estimated to exceed the level of no concern for nephrotoxicity set by EFSA (2012) in 16 % of pregnant women. No evidence was found for an association between higher maternal daily intakes of CIT, and duration of pregnancy, birth weight, birth length, and head circumference at birth.