Publications since the EFSA 2012 opinion

In this guide

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20. A literature search was undertaken to gather any data published since the EFSA opinion on CIT in 2012. The following sections summarise the information retrieved from the years 2012-2024.

Toxicokinetics

21. 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). A study in animals demonstrated differences in the toxicokinetic properties of CIT between pigs and chickens (Meerpoel et al., 2020b).

Toxicity

22. 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 did not show significant difference between species (Faisal et al., 2019). In the presence of albumin, the acute cytotoxic effects of both DH-CIT and CIT were significantly decreased on a Madin-Darby canine kidney (MDCK) cell line.

23. A repeat dose study by Jagdale et al. (2020) (conducted according to OECD 407 guidelines) which treated rats daily by gavage with CIT (25 µg/kg bw or 100 µg/kg bw) 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. These findings are in support of the NOAEL of 20 µg/kg bw reported by EFSA.

24. A 60-day study in rabbits suggested that at low concentrations, CIT (15 mg/kg feed) 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; abstract only).

Developmental and Reproductive toxicity

25. Since the 2012 EFSA opinion, limited data has been published on the reproductive and developmental effects caused by CIT. The doses at which effects were reported in the published studies were in exceedance of EFSA's level of no concern for nephrotoxicity.

26. A repeated oral dose toxicity study in female mice was carried out by exposing the animals to 1.25, 7.5, 15 and 30 ppm CIT for 70-90 days in drinking water (Hayashi et al., 2012). CIT did not produce any noticeable toxicity at any of the dose levels, except for an increase of both absolute and relative ovary weights accompanied by large follicles at ≥ 15 ppm (authors estimated this was equivalent to 2.25 mg/kg body weight/day).

27. In a one generation study by Singh et al. (2016, abstract only) male and female rats were administered 1, 3 and 5 ppm CIT in feed for 10 weeks before mating. The offspring were also fed CIT at the same doses until the age of six weeks. The authors concluded that the effects of CIT could be observed until the F1 generation in a dose-dependent manner and that apoptosis and oxidative stress played a role in CIT toxicity. CIT toxicity however did not lead to apoptosis and oxidative stress in male gonads until the F1 generation. As only the abstract was available it is not clear how the authors reached their conclusions.

28. Sharma et al. (2012) administered CIT (10 mg/kg feed) 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.
29. Newly fertilised zebrafish eggs were exposed to concentrations of 0.78-50 µM CIT before individuals reached free-feeding stage. (Csenki et al., 2021). This is whilst the zebrafish are still embryos prior to reaching the juvenile stage of development 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.

Genotoxicity

30. In vitro assays showed CIT to induce a dose dependent increase in micronuclei (MN) frequencies, chromosomal aberrations and sister chromatid exchanges (Anninou, 2014; Föllmann, 2014; Tsai, 2023: abstract only).

31. A series of in vivo studies by Kuroda (2013) in rats administered CIT by gavage at 20-40 mg/kg bw for a maximum of 28 days showed no evidence that chromosomal abnormalities, or genotoxic mechanisms were involved in CIT-induced renal carcinogenesis.

Carcinogenicity

32. 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; abstract only).

33. In vivo CIT showed evidence of promoting cell cycle progression when rats were administrated 20 and 40 mg/kg bw day CIT for 28 days (Kuroda et al., 2013). 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 of rats treated with CIT in the high dose group and the labelling index of proliferating cell nuclear antigen (PCNA)-positive cells was significantly increased at all doses. The mRNA expression analysis showed increases in Ccna2, Ccnb1, Ccne1, and its transcription factor E2f1 following treatment with all doses of CIT.

Immunogenicity

34. Limited data was available on the immunotoxicity of CIT since the EFSA opinion in 2012.

35. In in vitro mammalian cell assays CIT was reported to show evidence of immunomodulatory and immunotoxic effects (Sugiyama et al., 2013: abstract only; Islam et al., 2012; Xu et al., 2022).

36. In vivo, mice treated with CIT (1, 5, or 10 mg/kg bw) showed reduced levels of serum immunoglobulin M (IgM) and changes in the regulation of the different immune cell populations in the spleen, mesenteric lymph nodes and small intestine. The authors concluded that CIT has multiple immune modulatory effects in mice that may alter normal functions of immune system (Islam et al., 2012).