Toxicity - Citrinin

In this guide

13. CIT is acutely nephrotoxic in mice and rats, rabbits, pigs and poultry, causing swelling and eventual necrosis of the kidneys. CIT also affects liver function but to a lesser extent. Both in vitro and in vivo studies have provided clear evidence for reproductive and developmental toxicity of CIT.

Summary of EFSA 2012 opinion

14. In 2012, EFSA assessed the risks to public and animal health related to the presence of CIT in food and feed and concluded that the derivation of a health-based guidance value (HBGV) would not be appropriate, given the available data on genotoxicity and the limitations and uncertainties in the current database.

15. For compounds that are potentially genotoxic or carcinogenic EFSA recommends the use of the margin of exposure (MOE) approach. However, for CIT EFSA did not consider an MOE approach appropriate, due to the lack of human dietary exposure data. Instead, EFSA decided to characterise the risk of CIT and determine a level of no concern for nephrotoxicity in humans of 0.2 µg/kg bw per day. This was based on a no observed effect level (NOAEL) of 20 µg/kg bw per day determined from the 90 day study by Lee et al. (2010) in rats (see paragraph 25). A default uncertainty factor (UF) of 100 was applied, 10 for interspecies and 10 for interindividual variation.

16. EFSA concluded that a concern for genotoxicity and carcinogenicity cannot be excluded at the level of no concern for nephrotoxicity.

Toxicokinetic

17. The available information on CIT shows it is eliminated predominantly by renal excretion, approximately 75 % of radiolabelled citrinin (14C-citrinin) given by intraperitoneal dose was recovered in urine (Reddy et al., 1982).

18. 14C-CIT was administered to pregnant female rats by subcutaneous injection of a dose of 35 mg/kg bw on the 12th day of gestation. Elimination of 14C-CIT-derived radioactivity from plasma was found to be biphasic. The half-lives for the rapid (alpha) and slower (beta) phases of elimination were 1.95 hours and 39.7 hours, respectively. Approximately 74 % of the radioactivity appeared in the urine in the first 24 hours, with only 1.7 % and 1.4 % in the urine at 48 hours and 72 hours, respectively.

19. Toxicokinetic studies with oral administration of CIT were not available.

Acute toxicity

20. The acute lethal toxicity of CIT ranged from 19-134 mg/kg bw depending on species and route of administration.

21. The median lethal dose (LD50) for oral administration (105-134 mg/kg bw in mouse and rabbit) was higher than intravenous, intraperitoneal and subcutaneous administration. Intravenous administration was only documented in rabbit, but this resulted in the lowest LD50 (19 mg/kg bw). LD50 for subcutaneous and intraperitoneal administration was comparable for rat, mouse, guinea pig and rabbit (35-89 mg/kg bw).

22. The main changes in pathology were degeneration and necrosis of the kidneys in all species indicating nephrotoxicity.

Repeat dose and (sub)chronic toxicity

23. Repeat dosing studies confirmed the nephrotoxicity of CIT and highlighted the differences in susceptibility between species. Necropsy showed histopathological changes in the kidneys of all species tested (except hamsters), which were consistent with nephrotoxicity seen in the acute studies. Dogs were the most sensitive with intraperitoneal administration of 10 mg/kg bw/day for two weeks leading to spontaneous mortality after 7-11 days. Hamsters tolerated an

estimated 50 mg/kg bw/day in feed for two weeks with no clinical signs of toxicity, no gross lesions at necropsy and no histopathological changes.

24. Intraperitoneal administration of 50 mg CIT/kg bw for 2-4 days in guinea pigs led to mortality of all animals and histopathological changes were seen in the kidneys of the deceased guinea pigs (Carlton, 1978). An LD50 value of 43 mg/kg bw was determined in guinea pigs by Thacker et al. (1977) following the oral administration of CIT for 14 days.

25. A 90-day study with male Wistar rats by Lee et al. (2010), aimed to establish a safe concentration for dietary RMR as a food additive. CIT was given in the form of fermented RMR and contained different concentrations of CIT (1, 2, 10, 20 and 200 mg/kg). At the highest dose tested, no toxicologically significant alterations were observed, and the authors concluded that RMR containing CIT at a level of 200 mg CIT/kg RMR (equivalent to 20 µg CIT/kg bw per day) was not nephrotoxic. The authors therefore derived a NOAEL of 20 mg/kg bw per day in rats.

26. An 80-week feeding study exposed rats to CIT in the diet at about 70 mg/kg bw per day initially, however EFSA stated that as the body weight in the treated rats declined throughout the experiment, the estimation of the actual dose was difficult (Arai and Hibino, 1983). The study identified the kidney as the main target organ for toxicity and reported progressive histopathological changes and incidences of adenomas. EFSA concluded that given the observed high incidence of adenomas it cannot be excluded that carcinomas would have occurred if exposure time had been increased to the full length of a carcinogenicity study (at least 2 years).

Genotoxicity

27. Overall, EFSA concluded that the available literature data indicate that CIT is not mutagenic in conventional bacterial assays either with or without metabolic activation by S9 fraction from rat or human liver or rat kidney. Mutagenicity in the Ames test was only reported in one study when rat hepatocytes were used as activating system. In mammalian cells in vitro, CIT did not induce DNA single-strand breaks, oxidative DNA damage or sister chromatid exchanges (SCE) but induced micronuclei (MN), aneuploidy and chromosomal aberrations.

28. In vivo studies in mice exposed to CIT at concentrations of 5-20 mg/kg bw for eight weeks by oral administration showed the induction of chromosome abnormalities and hypodiploidy in bone marrow (Jeswal, 1996, abstract only

available).

Immunotoxicity

29. EFSA concluded that the data on immunotoxicity of CIT were incomplete and often non-specific, and therefore did not allow for a conclusive evaluation. EFSA further noted that the available studies were old and, in most cases, did not focus on immunology. A study carried out in beagles (20 and 40 mg/kg bw for 2 days administered in gelatine capsules and after that intraperitoneal because of profound emetic effects) report inconsistent changes in leukocyte counts (Carlton et al., 1974). EFSA considered that the results in this study were most likely related to dehydration of CIT treated animals than to any direct effect of CIT (20 and 40 mg/kg bw for 2 days administered in gelatine capsules and after that intraperitoneal because of profound emetic effects).

Developmental and reproductive toxicity

30. Data from in vitro and in vivo studies reported reproductive toxicity and teratogenic and embryotoxic effects of CIT. However, the in vivo studies also reported maternal toxicity, including nephrotoxicity, indicating that the reproductive, teratogenic and embryotoxic effects may be secondary to maternal toxicity. One study in male mice showed adverse effects on male reproductive organs and sperm quality when CIT was given intraperitoneally (0.0625, 0.625 or 6.25 mg/kg bw daily).

31. In mice which received an intraperitoneal dose of 10, 20, 30 or 40 mg CIT/kg bw on one of days 7-10 of gestation, there were no detectable adverse prenatal effects except at dose levels toxic to the dams. (Hood et al., 1976). At doses of 30 and 40mg/kg adverse effects on foetal growth and survival were observed.

32. A single subcutaneous dose of 35 mg citrinin/kg bw on days 3-15 of gestation was administered to rats. No skeletal malformations of the foetuses were observed (Reddy et al., 1982a). However, enlarged kidneys, internal hydrocephalus and cleft palates were reported. While 30-50 % of the pregnant dams died, the resorption rate of foetuses in the treated group was higher than in the controls. The authors concluded that citrinin was an embryocidal and fetotoxic agent, but that maternal toxicity potentially influenced the outcome of this study.

33. Kinetic investigations in pregnant rats provided no conclusive data about the percentage of CIT that crosses the placenta (Reddy et al., 1982b).

34. However, enlarged kidneys, internal hydrocephalus and cleft palates were reported. While 30-50 % of the pregnant dams died, the resorption rate of foetuses in the treated group was higher than in the controls. The authors concluded that CIT was an embryocidal and fetotoxic agent, but that maternal toxicity potentially influenced the outcome of this study.

35. In a series of experiments, the effect of CIT on pregnant Wistar rats and their offspring was investigated. CIT was administered at a concentration of 10 mg/kg feed (equivalent to approximately 1 mg/kg bw) during the gestation period (6 - 20 days post coitum). The dams showed mild maternal toxicity in the liver, intestines and kidneys. Maternal weight gain and feed intake were also reduced (Singh et al., 2007a, 2007b). In the same study, foetal resorption rate was increased and 6.8 % of the examined foetuses showed severe malformations, including internal hydrocephalus and notched and contracted kidneys. About 10 % of all foetuses were retarded with incomplete ossification of the skull bones. Histological investigations of the foetal kidneys showed tubular degeneration, medullar tubular necrosis and interstitial fibrosation (Singh et al., 2008).

36. EFSA concluded that the study performed by Singh et al. confirmed that CIT induces teratogenicity in rodent species. EFSA however noted that the doses at which effects were seen also induced maternal toxicity and that the extent of offspring exposure could not be determined in this study. Moreover, data on the actual exposure remain inconsistent, as all dams showed reduced feed intake at variable levels. Hence, the deviation from the targeted dose (1 mg/kg bw) was considerable and contributed to the uncertainty of this study.

37. The embryotoxic potential of CIT was also investigated in the chick embryo model (at 1-10 µg, injected subgerminally or intra-amniotically at different days of the egg incubation period) (Vesela et al., 1983). Effects on early embryonic development were observed including embryonic death (up to 60 %), and morphological alteration on embryos heads (exencephaly, micro-ophthalmia and cleft beak).

38. More recent in vitro studies with mouse embryonic cells confirmed the previous in vivo data. Blastocysts treated with CIT showed apoptosis and significant decreased implantation rates (Chan and Shiao, 2007). An increased rate of apoptosis was observed in mouse embryoblasts following ex vivo treatment with CIT at a concentration of 30 µg/mL (Chan, 2007). In further in vitro experiments, Chan (2008) observed a significant reduction in the rate of oocyte maturation, fertilization and embryonic development.

39. Qingqing et al. (2012) investigated the effects of CIT on the reproductive organs of male mice. Adult male mice (4 months of age) received an intraperitoneal injection of CIT (0.0625, 0.625 or 6.25 mg/kg bw), daily for 7 days. CIT significantly increased the relative reproductive organ weights and decreased sperm count at all doses. Histopathological changes in the testes were also seen. A significantly lower pregnancy rate was observed when females were mated with the previously exposed males and no embryos occurred in the females mated with males given the highest dose of 6.25 mg/kg bw.

Publications since the EFSA 2012 opinion

40. A literature search was undertaken between 2012 and 2024 to gather any data published following the EFSA opinion on CIT in 2012. The following sections summarise the information found.

Toxicokinetic

41. A study by Degen et al. (2018) exposed two healthy female volunteers to doses of CIT (0.045-0.114 µg/kg) below the NOAEL for nephrotoxicity set by EFSA (2012), on two occasions, several weeks apart. CIT was dissolved in ethanol and added to a soft drink. Urinary excretion showed that ingested CIT undergoes conversion to dihydrocitrinone (DH-CIT) which is then excreted in the urine along with the remaining parent compound. The cumulative urinary excretion within 24 hours was between 32.9% and 70.8% of the sum of CIT and DH-CIT. The median half-life in urine was 6.7 hours for CIT and 8.9 hours for DH-CIT.

42. Meerpoel et al. (2020b) carried out toxicokinetic analysis of CIT in pigs and broiler chickens. CIT (50 µg/kg bw for pigs, 250 µg/kg bw for chickens) was administered to four animals of each species by oral gavage or intravenous injection. The author states that that oral bioavailability for CIT was complete for broiler chickens (113–131%) and 37-44% in pigs with CIT more rapidly absorbed in pigs (Tmax = 0.92 hours) compared to broiler chickens (Tmax = 7.33 hours). Elimination of CIT was slower in pigs (T1/2el = 26.81 hours after intravenous administration) compared to chickens (T1/2el = 1.97 hours after intravenous administration) with the clearance rate higher in broiler chickens (Cliv=9.87 mL/h/kg for pigs versus Cliv = 863.09 mL/h/kg for broilers). The results also showed that the volume of distribution differed significantly between pigs (Vd = 0.30 L/kg after intravenous administration) and chickens (Vd = 2.46 L/kg after intravenous administration).

In vitro

43. Chinese hamster lung fibroblast cells were treated with concentrations of either CIT (0.1–100 µM CIT) or DH-CIT (0.1–300 µM) (Föllmann et al., 2014). The cytotoxic potency of DH-CIT (IC50 of 320/200 µM) after treatment for 24 and 48 hours was lower compared with CIT (IC50 of 70/62 µM). The authors concluded that the lower cytotoxicity of DH-CIT indicated it was a detoxification step.

44. The interaction between DH-CIT and albumin did not show significant species differences when tested with human, bovine, porcine, and rat albumins (Faisal et al., 2019). The presence of albumin however significantly decreased the acute in vitro cytotoxic effects of both DH-CIT and CIT on a MDCK cell line.

Genotoxicity

45. Föllmann et al. (2014) carried out an in vitro micronucleus (MN) assay on V79 cells (Chinese hamster lung fibroblasts) with 10–100 µM CIT and 1–500 µM DH-CIT. The results indicated that CIT induced a concentration-dependent increase in MN frequencies at concentrations ≥ 30 µM, while its metabolite DH-CIT showed no genotoxic effect up to 300 µM.

46. Anninou et al. (2014) investigated the genotoxicity of CIT on a Hep3B cell line at concentrations below those detected in human serum (10-12–10-6 M). Results showed enhanced sister chromatid exchanges (SCEs) at low (pM) concentrations, but not in a dose dependent manner.

47. A study by Tsai et al. (2023; abstract only), exposing human renal proximal tubule epithelial cells (hRPTECs) to CIT (10 and 20 µM) over 3 days showed chromosomal aberrations. Exposure of human embryonic kidney 293 (HEK293) cells to CIT at the same concentration for 3 and 30 days significantly promoted mitotic spindle abnormalities, wound healing, cell migration, and anchorage-independent growth. The authors concluded that both short- and long-term CIT exposure activated cancer and cell cycle-related signalling pathways.

Acute toxicity

Reproductive and developmental toxicity

48. Newly fertilised zebrafish eggs were exposed to concentrations of 50, 25, 12.5, 6.25, 3.125, 1.56, and 0.78 µM CIT before individuals reached free-feeding stage (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.

Short term repeat dose toxicity

49. In a repeat dose study by Jagdale et al. (2020) (according to OECD 407 guidelines) 25 rats of each sex were divided equally into five dosing groups. Animals were treated daily by gavage for 28 days with either a vehicle control (corn oil with 0.4% DMSO) or CIT at 25 µg/kg bw or 100 µg/kg bw. Results showed no abnormal clinical signs during the experimental period. There were no significant histological changes in any of the low dose (25 µg/kg bw) group animals when compared to control animals. Adverse histopathological changes however were observed in the kidney, and the spleen at the high dose (100 µg/kg bw).

Reproductive and developmental toxicity

50. CIT (10 mg/kg feed) was administered orally to pregnant Wistar rats from gestational day (GD) 6-20 (Sharma et al., 2012). Fluorescence activated cell sorter analysis was performed on liver, kidneys and spleen tissue samples of dams, and results showed a significant increase in the percentage of apoptotic cells in liver, kidneys and spleen. Results from the liver and kidney of foetuses showed a significant increase in the percentage of apoptotic cells in kidneys.

51. A study by Li et al. (2023) looked at the effects of CIT on male reproductive toxicity, by exposing rats to 0, 1, 2.5, and 5 mg/kg CIT by gavage from postnatal days (PND) 21-28. At a dose of 5 mg/kg CIT a significant decreased in serum testosterone levels was observed, while an increase in serum luteinizing hormone (LH) and follicle stimulating hormone (FSH) levels could be seen. In males, LH stimulates Leydig cell production of testosterone and FSH enhances the production of androgen-binding protein by the Sertoli cells which critical for the initiation of spermatogenesis. The authors suggested that CIT inhibits Leydig cell development at multiple levels via different mechanisms with oxidative stress partially playing a role.

Genotoxicity

52. Kuroda et al. (2013) performed three in vivo genotoxicity assays in male F344 gpt delta rats: an in vivo reporter gene mutation assay using the kidneys, an in vivo comet assay, and an in vivo micronucleus (MN) assay using the kidneys

and bone marrow of F344 rats, respectively. Groups of five rats were administered CIT by gavage at 20-40 mg/kg bw per day for 2-28 days depending on the assay. Results of the in vivo reporter gene mutation assay (exposure 28 days) showed no significant increases in gpt or Spi− mutant frequencies (MF) in DNA extracted from the kidney cortices, suggesting that genomic mutations were not induced. In the comet assay (exposure 2 days), no changes were seen in the kidney cortices, indicating that no DNA damage occurred. The MN assay (exposure 2 days) also showed no significant increase in the micronucleated polychromatic erythrocytes/polychromatic erythrocytes ratio at any dose of CIT. The authors therefore concluded that chromosomal abnormalities or genotoxic mechanisms were not significantly involved in CIT-induced renal carcinogenesis.

Carcinogenicity

53. CIT was administrated at 20 and 40 mg/kg bw day to gpt delta rats by gavage for 28 days to study its proliferative effects in the kidney (Kuroda et al., 2013). Due to decreases in body weight the maximum dose of 40 mg/kg was decreased to 30 mg/kg from day four. Regenerative tubules were observed in the high (40/30 mg/kg bw) dosing group, not in the low dosing group (20 mg/kg bw group). In the kidney cortex of rats treated with CIT, the labelling index of proliferating cell nuclear antigen (PCNA)-positive cells was significantly increased at all doses compared with that of the control group. The mRNA expression analysis showed increases in Ccna2, Ccnb1, Ccne1, and its transcription factor E2f1 following treatment with all doses of CIT. The authors therefore concluded that CIT promoted cell cycle progression.

(Sub) Chronic toxicity

Nephrotoxicity

54. In a study by Kumar et al. (2014; abstract only) rabbits were fed with diets containing OTA (0.75 mg/kg feed), CIT (15 mg/kg feed) and OTA + CIT (0.75 and 15 mg/kg feed, respectively) daily, up to 60 days. The results suggested that low concentrations of OTA and CIT either alone or in combination 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.

Reproductive and developmental toxicity

55. A 70-day repeated oral dose toxicity study in female mice was carried out by exposing the animals to 1.25 and 7.5 ppm CIT in drinking water (Hayashi et al., 2012). CIT did not produce any noticeable toxicity in the kidneys, liver, and female genital organs/tracts, except for a slight increase of relative ovary weight. This was followed up with a 90-day repeated oral dose toxicity study, with increased CIT doses of 15 and 30 ppm in drinking water. The results again showed no toxicity in the kidneys, liver, and female genital organs/tracts, except for an increase of both absolute and relative ovary weights accompanied by large follicles at ≥ 15 ppm. On the basis of these findings, the authors determined a lowest-observable-adverse-effect level (LOAEL) of 15 ppm (2.25 mg/kg body weight/day) for CIT.

56. In a one generation study by Singh et al. (2016, abstract only) male and female rats were divided into dosing groups of 1, 3 and 5 ppm CIT in feed. After 10 weeks male and female rats were mated, and pregnant females were allowed to give birth to offspring (F1 generation) naturally. The offspring were also fed CIT in the above-mentioned doses till the age of 6 weeks and then killed. The authors concluded that the effects of CIT were observed until the F1 generation in a dose-dependent manner and apoptosis and oxidative stress play a role in CIT toxicity. CIT toxicity however did not lead to apoptosis and oxidative stress in male gonads until F1 generation. As only the abstract was available it is not clear how or whether the authors reached the conclusion that effects seen in the F1 generation were a result of exposure of the parent generation, given that offspring were also dosed with CIT.

Human Exposures

Epidemiological studies

57. 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).

58. A study in Nigerian children showed DH-CIT to be present in breastmilk at concentrations of 14.0 –59.7 ng/L. DH-CIT was also measured in the urine of infants who were exclusively breastfed at levels of 5–944 ng/L compared to levels of 5–1,377 ng/L in infants who were not exclusively breastfed (Ezekiel et al., 2022).

59. 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). A provisional daily intake for CIT was calculated and exceeded the level set by EFSA (0.2 µg/kg bw) in 23.3% and 11.9% of children and infants, respectively. CIT was detected in 61% of the urine samples collected from pregnant women (n=447) and dietary exposure to CIT was of public health concern in 16% of the pregnant women. The results of these studies indicated frequent co-exposure to nephrotoxic mycotoxins that varied between the cohorts and regions in Bangladesh. 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. The authors noted that there was large uncertainty regarding the relationship between maternal dietary exposure to CIT and pregnancy loss, preterm birth, born small for gestational age, and small-vulnerable newborns.

60. A study by Narváez et al. (2021) measured CIT and its metabolite DH-CIT in 300 urine samples in the Italian population. CIT was detected in 47% of the samples (n = 300) at concentrations up to 4.0 ng/mg creatinine (Crea) (mean value = 0.29 ng/mg Crea), whereas DH-CIT was detected in 21% of samples (n = 300) up to 2.5 ng/mg Crea (mean value = 0.39 ng/mg Crea). The average exposure to CIT from food ranged from 8% to 40% of the NOAEL in rat for nephrotoxicity (0.2 µg/kg bw per day), set by EFSA (2012), with children being the group with the highest exposure. Four individuals exceeded this NOAEL.

Balkan Endemic nephropathy

61. Balkan endemic nephropathy (BEN) is a familial, slowly progressive, chronic renal disease with insidious onset in the fifth decade of life and terminal renal failure in the sixth or seventh decade (Pavlović, 2013). The pathology of BEN is characterized by a progressive atrophy and sclerosis of all structures of the kidney, and it shares similarities with tubulointerstitial kidney diseases.

62. The occurrence of BEN has been recorded with a high prevalence rate in Serbia, Bulgaria, Romania, Bosnia and Herzegovina and Croatia. The most prominent features of the disease are its endemic nature, the long latency period before development of the disease, familial clustering of the disease and the high incidence of upper urothelial cancer (UUC).

63. Hypotheses relating to the aetiology of BEN include both exogenous (including lead and mycotoxins) and endogenous (including genetic predisposition and viral disease) factors and Pavlović et al. concluded that it is likely multifactorial. The exact aetiology remains unknown.

64. CIT is suspected to be one of the aetiological agents of BEN and CIT-specific DNA adducts were detected in the renal tumours of patients with BEN (Pfohl-Leszkowicz et al., 2007). Studies have indicated that CIT is present in considerable amounts in the diets of people living in Balkan endemic nephropathy areas (Petkova-Bocharova et al., 1991; Vrabcheva et al., 2000).