Toxicity

In this guide

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6. CIT is acutely nephrotoxic in mice and rats, rabbits, pigs and poultry, causing enlargement 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 (EFSA, 2012).

Previous assessments

EFSA 2012 opinion

7. In 2012, the European Food Safety Authority (EFSA) assessed the risks to public and animal health related to the presence of CIT in food and feed.

Toxicokinetics

8. 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). Toxicokinetic studies with oral administration of CIT were not available.

Toxicity

9. 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 were degeneration and necrosis of the kidneys in all species indicating nephrotoxicity. 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 the acute signs observed.

Genotoxicity and Carcinogenicity

10. 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.

11. CIT is acutely nephrotoxic in mice and rats, rabbits, pigs and poultry, causing enlargement 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 (EFSA, 2012).

12. In 2012, the European Food Safety Authority (EFSA) assessed the risks to public and animal health related to the presence of CIT in food and feed.

Immunotoxicity

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

Developmental and reproductive toxicity

14. Data from *in vitro* and *in vivo* studies reported reproductive toxicity and teratogenic and embryotoxic effects of CIT (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.

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Health based guidance value

16. EFSA concluded that the establishment 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.

17. 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. A level of no concern for nephrotoxicity is less secure than a HBGV and is a concentration below which there is no appreciable concern for nephrotoxic effects. This level does not specifically address other end points.

18. The level of no concern was based on a no observed adverse effect level (NOAEL) of 20 µg/kg bw per day determined from a study in rats by Lee et al. (2010). In this study, CIT was given in the form of fermented RMR containing different concentrations of CIT (1, 2, 10, 20 and 200 mg/kg) and at the highest dose tested (equivalent to 20 µg CIT/kg bw per day) no toxicologically significant alterations were observed for any dose group. EFSA applied a default uncertainty factor (UF) of 100 for interspecies and interindividual variation.

19. EFSA concluded that a concern for genotoxicity and carcinogenicity could not be excluded at the level of no concern for nephrotoxicity.

Publications since the EFSA 2012 opinion

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

Toxicokinetics

21. EFSA concluded that the establishment 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.

22. The toxicity study by Sharma (2012) (see paragraph 29) indicates that CIT can cross the placenta.

Toxicity

23. The level of no concern was based on a no observed adverse effect level (NOAEL) of 20 µg/kg bw per day determined from a study in rats by Lee et al. (2010). In this study, CIT was given in the form of fermented RMR containing different concentrations of CIT (1, 2, 10, 20 and 200 mg/kg) and at the highest dose tested (equivalent to 20 µg CIT/kg bw per day) no toxicologically significant alterations were observed for any dose group. EFSA applied a default uncertainty factor (UF) of 100 for interspecies and interindividual variation.

24. 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 support the NOAEL of 20 µg/kg bw reported by EFSA.

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

Developmental and Reproductive toxicity

26. 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.

27. The toxicity study by Sharma (2012) (see paragraph 29) indicates that CIT can cross the placenta.

28. 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). 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.

29. 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. The effects caused by CIT administration on dams and foetuses were not reported, but toxicity as a result of apoptotic cells in the kidneys is inferred by the authors.

30. 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).

Genotoxicity

31. 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.

32. 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

33. In a one generation study by Singh et al. (2016) 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 renal toxicity. CIT toxicity however did not lead to apoptosis and oxidative stress in male gonads including the F1 generation.

34. 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. The effects caused by CIT administration on dams and foetuses were not reported, but toxicity as a result of apoptotic cells in the kidneys is inferred by the authors.

35. Cyclin B1 (CCNB1 gene) is also a highly conserved cyclin family protein that is ubiquitously expressed in humans, and which is purportedly involved in regulating tumour epithelial–mesenchymal transitions and metastasis. It plays a key role in controlling the G1–S and G2–M cell cycle transitions.

Immunogenicity

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

37. 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).

38. *In vivo,* mice treated 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. The authors concluded that CIT has multiple immune modulatory effects in mice that may alter normal functions of immune system and induced T-cell-specific lymphoproliferative capacity (Islam et al., 2012).

Epidemiological studies

39. 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 both doses. The mRNA expression analysis showed increases in Ccna2, Ccnb1, Ccne1, and its transcription factor E2f1 following treatment with all doses of CIT. Authors suggested that this indicated induction of cell cycle progression at all tested doses of CIT.

40. Cyclin B1 (CCNB1 gene) is also a highly conserved cyclin family protein that is ubiquitously expressed in humans, and which is purportedly involved in regulating tumour epithelial–mesenchymal transitions and metastasis. It plays a key role in controlling the G1–S and G2–M cell cycle transitions.

41. Overall, the new data published since the 2012 EFSA opinion supports previous findings or adds to the overall knowledge base of CIT. CIT is acutely nephrotoxic, and both *in vitro* and *in vivo* studies have provided provide some evidence that dietary exposure to citrinin mat cause reproductive and developmental toxicity, although most of the effects observed were at maternally toxic doses.

42. The COT agrees with EFSA that a HBGV cannot be set and that it was appropriate to use a level of no concern for nephrotoxicity to characterise the risk of CIT to consumers. The doses administered in the available reproductive and developmental studies were higher than the level of no concern for nephrotoxicity, and so this level would be adequately protective for maternal, reproductive and developmental toxic effects.

Exposure Assessment

43. Exposure to CIT was determined for women of child-bearing age (16-49 years), using consumption data from the National Diet and Nutrition Survey (NDNS) and occurrence data from the 2014 Total Diet Study (TDS) (Bates et al., 2014, 2016, 2020; Roberts et al., 2018, FSA, 2014).

44. Occurrence data from all food samples analysed for CIT were below the limit of quantification (LOQ) and the exposures calculated are based on the lower bound (LB) and upper bound (UB) values. As the LB is zero for a commodity, it cannot be determined whether a commodity makes a contribution to the overall exposure.

45. Mean total exposure to CIT for women of child-bearing age ranged from 0-17 ng/kg bw/day, whilst exposure in high consumers (97.5^{th} percentile) ranged from 0-43 ng/kg bw/day. The food groups with the highest UB values were tea with a mean value of 6.2 ng/kg bw/day and a 97.5^{th} percentile value of 23 ng/kg bw/day; instant coffee with a mean value of 2.6 and 97.5^{th} percentile value of 17 ng/kg bw/day; wine with a mean value of 1.0 ng/kg bw/day, and 97.5^{th} percentile value of 6.5 ng/kg bw/day.

46. The carryover of CIT into animal products was not included in the exposure assessment but would not be expected to significantly add to the exposure under normal, non-experimental, circumstances.

Risk characterisation

47. CIT is nephrotoxic, causing enlargement and eventual necrosis of the kidneys, and in some studies was also reported to affect liver function. Exposure to CIT has also been associated with reproductive toxicity and teratogenic and embryotoxic effects albeit usually at doses that were maternally toxic.

48. Based on the data available, including data published since the EFSAs opinion, the COT did not think it appropriate to establish a HBGV but continued to use EFSA's approach, applying a level of no concern for nephrotoxicity in humans of 0.2 µg/kg bw per day.

49. While a number of studies reported developmental and reproductive toxicity of CIT it is not clear whether these effects might be secondary to maternal toxicity. A study reported by EFSA in 2012 failed to determine the amount of CIT that would cross the placenta, and no metabolites of CIT were detected in the foetus. However, as the doses administered in the available reproductive and developmental studies were higher than the level of no concern for nephrotoxicity, the COT considered the level of no concern for nephrotoxicity to be adequately protective for maternal, reproductive and developmental toxic effects.

50. In 2012, EFSA did not consider there to be sufficient data to conclude on the immunotoxic effects of CIT. While some additional data has been published since EFSA's opinion, the database is still very limited, and a conclusive assessment cannot be carried out.

51. The available data demonstrates that citrinin does not cause gene mutations but may have a thresholded effect on microtubules and/or spindle assembly. However, due to the limitations in the database a risk of genotoxicity and carcinogenicity cannot be excluded although citrinin showed no evidence of DNA-reactive mutagenicity.

52. Mean and 97.5th percentile total estimated exposures for CIT were 0-17 and 0- 43 ng/kg bw respectively and are below the level of no concern for nephrotoxicity set by EFSA. Hence, the estimated exposures are not of toxicological concern for nephrotoxicity and reproductive and developmental effects, but carcinogenicity and genotoxicity cannot be excluded.

53. It should be noted that the TDS data used to calculate exposure are from 2014 and changes in the prevalence of citrinin may have occurred since then. Dietary patterns may also have changed, for example the increased consumption of plant-based drinks, and vegan/vegetarian diets, which may not be

fully represented in the data.

54. The current assessment was based on consumption data from the NDNS for women of maternal/childbearing age and therefore may not be representative of maternal diet. In addition, the NHS recommends that those who are pregnant or planning to become pregnant should not drink alcohol. The inclusion of the UB values for wine, beer, alcopops and cocktails in the assessment may therefore lead to an over estimation of exposure when considering pregnant women.