Toxicology

In this guide

9. EFSA carried out a full assessment of citrinin in 2012. Their assessment has been used as a baseline point for the following hazard characterisation. In addition, a literature search covering the period 2012-2024 was carried out, ensuring that relevant data published after the EFSA assessment were also considered here. The key studies used in the RIVM risk assessment (2017) were retrieved as part of this literature search. The following sections therefore summarise all relevant toxicological information up to 2024.

Toxicokinetics

10. The available information on citrinin showed it is eliminated predominantly by renal excretion; approximately 75 % of radiolabelled citrinin (14C-citrinin) given to pregnant Spraque-Dawley rats by subcutaneous administration was recovered in urine (Reddy et al., 1982a). A study by Meerpol et al. (2020b) demonstrated differences in the toxicokinetic properties of citrinin 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 cytotoxicity of the metabolite dihydrocitrinone was less than that of citrinin (Föllmann et al., 2014) while the interaction of dihydrocitrinone with albumin from different species in vitro did not differ significantly (Faisal et al., 2019).

12. 12. A study in human volunteers demonstrated that ingested citrinin undergoes conversion to dihydrocitrinone, 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 citrinin can cross the placenta (discussed further in the Developmental and reproductive toxicity section, see paragraph 31).

Experimental toxicity

Genotoxicity

14. EFSA concluded that the available data indicated that citrinin was 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 a single study when rat hepatocytes were used as the activating system (Sabater-Vilar et al., 1999). In mammalian cells *in vitro*, citrinin did not induce DNA single-strand breaks, oxidative DNA damage or sister chromatid exchanges but did induce micronuclei, aneuploidy and chromosomal aberrations.

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

16. In vivo, citrinin induced chromosome abnormalities and hypodiploidy in the bone marrow of mice exposed to oral doses of 5-20 mg/kg bw for eight weeks (Jeswal, 1996).

Carcinogenicity

17. Fischer 344 rats were fed diet containing citrinin at 0.1 % (correspond to an intake of 1000 mg/kg diet or exposures equivalent to approximately 70

mg/kg bw based on 20 g feed intake) 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. In a series *of in vivo* studies, Kuroda et al. (2013) administered citrinin to male Fischer 344 rats by gavage at 20-40 mg/kg bw/day for a maximum of 28 days. The higher dose, which is 2000 times higher than the POD selected by EFSA , is identified by the authors as “the maximal tolerated dose and a nearly carcinogenic dose”. The maximum dose was decreased to 30 mg/kg from day four due to decreases in body weight (bw). Regenerative tubules were observed in the kidney cortex in the high dose group and cell proliferation was significantly increased at both doses. The authors suggested that the increase in cyclin encoding genes (*Ccna2, Ccnb1, Ccne1*) and 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 dihydrocitrinone and citrinin were significantly decreased in Madin-Darby canine kidney (MDCK) epithelial cell line, in the presence of albumin (Faisal et al., 2019).

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

21. In a 90-day study in male Wistar rats by Lee and Pan (2010), citrinin was given in the diet in the form of fermented RMR containing different concentrations of citrinin (1, 2, 10, 20 and 200 mg/kg). At the highest dose tested (stated by EFSA to be equivalent to 20 µg/kg bw/day) no toxicologically significant alterations in body weight gain, daily feed intake, organ weight and serum biochemistry or histopathology of livers and kidneys were observed.

22. In a repeat dose study, Jagdale et al. (2020) treated male and female Wistar rats with 25 µg/kg bw/day or 100 µg/kg bw/day citrinin by gavage for 28 days. Adverse histopathological changes were reported in the kidney and spleen at the higher dose. No significant histological changes were reported in animals dosed with 25 µg/kg bw/day.

23. A 60-day study in rabbits suggested that at low concentrations, citrinin (15 mg/kg feed, equivalent to 0.45 mg/kg bw/day; EHC 240) induced lipid peroxidation and apoptosis in a time-dependent manner in the kidney; this, 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 citrinin 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 citrinin have been published (Sugiyama et al., 2013: abstract only; Islam et al., 2012; Xu et al., 2022).

26. When immunoglobulin (Ig) levels were measured in mice treated *in vivo* with citrinin (1, 5, or 10 mg/kg bw/day, by gavage) for 14 days, a dose-dependent reduction in IgM was observed in the absence of significant changes in IgA, IgE and IgG (Islam et al., 2012). 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/day. *Ex vivo* exposure of primary splenocytes and mesenteric lymph node cells demonstrated induction of apoptosis in the mice spleen, lymph nodes and Peyer’s patches, which authors commented was through alteration of *Bax/Bcl-2* expression.

Developmental and reproductive toxicity

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

28. Toxicokinetic investigations in pregnant Charles River CD1 rats provided no conclusive data about the percentage of citrinin that crosses the placenta (Reddy et al., 1982b). Therefore, EFSA could not determine the extent to which 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 citrinin; however, where such effects were observed the doses administered were above EFSA's level of no concern for nephrotoxicity.

30. A repeated oral dose toxicity study by Hayashi et al. (2012) exposed female BALB/c mice to 0, 1.25 or 7.5 ppm citrinin 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 BALB/c mice to 0, 15 or 30 ppm citrinin 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 bw/day).

31. Singh et al. (2012) administered citrinin (10 mg/kg feed, equivalent to 1 mg/kg bw/day; EHC 240) to pregnant Wistar rats from gestational day 6-20. A significant increase in the percentage of apoptotic cells in kidneys of dams and foetuses as observed. Toxicity, indicated by the presence 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) 2014). male and female Wistar rats were administered citrinin via the diet (1, 3 and 5 mg/kg citrinin in feed, equivalent to 0.1, 0.3 and 0.5 mg/kg bw/day; EHC 240) for 10 weeks prior to mating, 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 citrinin before individuals reached the free-feeding stage, i.e. while the zebrafish were still embryos prior to reaching the juvenile stage of development.

(Csenki et al., 2021). No mortalities occurred, but exposure to 50 µM citrinin led to pericardial oedema, blood accumulation, incorrect heart looping, and reduced size of cardiac chambers.

Epidemiological studies

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

35. Citrinin and dihydrocitrinone 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). Citrinin 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).

36. Three biomonitoring studies were carried out to measure the concentration of citrinin and dihydrocitrinone in pregnant women, infants and children in Bangladesh (Ali and Degen, 2020; Kyei et al., 2022, 2023). Citrinin was detected in 61 % of the urine samples collected from pregnant women and dietary exposure to citrinin, 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 citrinin, and duration of pregnancy, birth weight, birth length, and head circumference at birth.

Other human studies

37. EFSA considered two reports of (accidental) human exposure (Hetherington and Raistrick, 1931; Ambrose and DeEds, 1946); however, both focussed on inhalation of citrinin powder. EFSA drew no conclusions from these studies, and the effects were not considered to be relevant to the maternal diet due to the route of exposure.