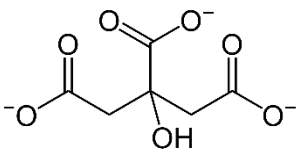
Citrate: Brief profile on systemic toxicological effects

1. Identification



For information on background, scope and use of this document, see accompanying cover-note!

Name: citrate (anion)
Chemical formula: $C_6H_5O_7^{3-}$
Molecular mass: 189.1 g/mol

The name "citrate(s)" is used for anions of citric acid, as well as for salts containing such anion(s). Esters of citric acid can also be named citrate. Citric acid is a polyprotic acid so that three anionic forms exist. In aqueous solutions with neutral pH, the predominant form is the triple-anion as shown above. In this document the term citrate usually refers to either of the three anionic forms, or to the solid salts of citric acid.

2. Introductory remarks

The aim of this summary on the systemic toxicity of "citrate" is to assist the assessor of health hazards of citrate-containing substances ("citrate as a counter-ion"), in determining whether the citrate moiety contributes to the overall toxicity of the substance. Ions such as citrate are not defined as a substance under REACH since they are always associated with a counterions to maintain electrical neutrality (formation of "salts"). The toxicology of such salts is always a combination of both ions, so that toxicological information is – in most cases - not available for the ion on its own. In the case of citrate, the corresponding acid citric acid is a solid as well, used in many applications in a similar function as the common inorganic citrate salts, e.g. as food additives. Toxicological data may be available for the salts as well as the acid itself. Because citric acid is polyprotic, various salts such as mono-, di- or trisodium citrate exist. The purpose of this document is not to discuss primary literature or studies in detail. Instead, reference is made to most published authoritative reviews (e.g. European Food Safety Authority (EFSA), WHO or OECD).

Citric acid and/or several citrate salts are authorised as minerals for addition to food, as food additives and in food supplements (Annex II of the Regulation (EC) No 1925/2006, Annex II of Directive 2002/46/EC and Regulation (EC) No 1333/2008). EFSA is currently in the process of re-evaluating various already authorised food additives, including citrates[1]. However, as of today (22 May 2020), the re-evaluation has apparently not been concluded or at least not yet been published. A previous comprehensive toxicological assessment by EFSA on citrates could not be found. The joint FAO/WHO Expert Committee on Food Additives (JECFA) has not conducted a full review on citric acid and its salts since 1973[2]. This 1973 review is very brief, refers to even older data and is thus not considered further here. Likewise, citric acid and various citrate salts are Generally Recognized As Safe (GRAS) food substances according to US FDA (1977, not considered further here, as also considered outdated).

[1] See e.g. http://www.efsa.europa.eu/en/consultations/call/180122, Table 2 on "Remaining food additives to be re-evaluated according to Regulation (EU) No 257/2010"
In a 2016 opinion on the safety of trimagnesium dicitrate as a food additive, the completion of the re-evaluation was anticipated for the end of 2018. (EFSA; 2016)

[2] http://www.inchem.org/documents/jecfa/jecmono/v05je24.htm

The most recent relevant published authoritative review on citric acid appears to be the 2013 "Human health tier II assessment" under the Australian NICNAS scheme[3], which however relies considerably on an OECD SIDS Initial Assessment Report of 2001[4].

Therefore, for the time being, this brief profile on systemic toxicological effects for citrate as a counter-ion frequently cites NICNAS (2013) and/or OECD (2001). With regards to systemic toxicological effects, toxicological data on citric acid can be used as a surrogate for citrate anions. **It is suggested to update this document if/when the ongoing EFSA re-evaluation on citric acid and citrates becomes available**.

3. Natural occurrence, physiological function and dietary intake

Citric acid is a natural substance that appears as an intermediate in the basic physiological citric acid cycle (Krebs cycle) in every eukaryotic cell (including human cells). It is also present naturally in food, for example, in citrus juices. Recent information on the actual human intake of citrate(s) via food (natural content or added) was not sought for this brief profile (expected to be gathered by EFSA as part of the ongoing re-evaluation).

4. Toxicokinetics

"In human (as well as in animal and plant) physiology, citric acid is a very common intermediate in one of the central biochemical cycles that takes place in every cell. Thus, in humans approximately 2 kg of citric acid are formed and metabolised every day. This physiological pathway is very well developed and capable of processing very high amounts of citric acid as long as it occurs in low concentrations. Part of the circulating (mainly metabolic but also ingested) citric acid is excreted in urine, with 24-hour urine reference values between 1.5 and 3.68 mmol, corresponding to 0.29–0.71 g citric acid excreted per person per day (OECD, 2001)." (cited from NICNAS, 2013)

5. Toxicological effects

NICNAS (2013) concluded that critical health effects for citric acid are local effects (skin, eye and respiratory irritation). These effects are not applicable to citrate anions. Pending the ongoing re-evaluations on the safety of citrates, EFSA's Scientific Committee on Food emphasised in 2016 that citrate has a well-established role as an intermediate metabolite in the citric acid cycle and as a natural component of food and confirmed the 'ADI not specified' established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1974 (EFSA, 2016).
The following sub-chapters mostly cite data on citric acid.

5.1. Acute toxicity

Citric acid is of low acute oral toxicity in animal tests following oral exposure. The median lethal dose (LD50) in rats ranges from 3000 to 12000 mg/kg bw. Observed sub-lethal effects included physiological disturbances (acidosis and calcium deficiency), while high doses caused nervous system effects as well as severe damage to the stomach mucosa (OECD, 2001).
Citric acid is of low acute toxicity in rats, with a median lethal dose (LD50) greater than 2000 mg/kg bw, following dermal exposure in tests conducted in accordance with OECD Test Guideline 402 (REACH). No acute inhalation toxicity data on citric acid are available. (adopted from NICNAC, 2013).

[3] https://www.nicnas.gov.au/chemical-information/imap-assessments/imap-group-assessment-report?assessment_id=198

[4] https://hpvchemicals.oecd.org/ui/handler.axd?id=ff78c453-36c1-430d-9034-63e15899d24b

5.2. Sensitisation

Patch testing of 60 eczema patients with 2.5 % citric acid in petrolatum did not produce any irritant or allergic reactions (OECD, 2001). Citric acid/citrate are also not considered as sensitising based on their natural occurrence in the human body and their physiological function.

5.3. Repeated dose toxicity

"A 2-year chronic oral study in rats being given 5 % (approximately 2000 mg/kg bw/d) or 3 % citric acid in feed (approximately 1200 mg/kg bw/d) found slightly decreased growth in the higher dosage group but no tissue abnormalities in the major organs. A NOAEL of 1200 mg/kg bw/d was determined. Similarly, NOAELs of 1500 mg/kg bw/d (rabbit) and of 1400 mg/kg bw/d (dog) have been determined for citric acid." Citric acid is not considered to cause serious damage to health from repeated oral exposure (OECD, 2001; cited from NICNAS, 2013).

No data are available on repeated dose toxicity via the dermal or inhalation route.

5.4. Mutagenicity/genotoxicity

"Citric acid was not found to be mutagenic or genotoxic in several in vitro and in vivo tests including bacterial mutation assays with and without metabolic activation, in vitro chromosomal aberration assays and a dominant lethal assay with male rats being treated up to 3000 mg/kg/d for 5 days." (OECD, 2001; cited from NICNAS, 2013).

5.5. Reproductive toxicity

OECD (2001) has summarised various studies on the possible effects of citric acid on reproductive and developmental toxicity. These studies data back as far as to the 1950s, and their reliability could not always be assessed by OECD. However, the database includes a 2-generation reproductive toxicity study in rats ("reliable with restrictions"), as well as prenatal developmental toxicity studies in mice, hamsters and rabbits. Based on the available information, no relevant adverse effects on reproduction or prenatal development were observed at doses up to 7500 mg/kg_{bw}/day. NICNAS (2013) concluded that no hazard classification for reproductive or developmental toxicity is required.

5.6. Carcinogenicity

"In a study with only 20 male rats receiving up to 5 % citric acid in the feed (approximately 2000 mg/kg bw/d) for 2 years no evidence of carcinogenicity was reported. In a further study with rats fed 1.7 % sodium citrate (approximately 740 mg/kg bw/d) for 8 weeks no increase in DNA synthesis, a measure of cell proliferation, in the bladder epithelium was found. Various studies concluded that citric acid does not act as a tumour promoter" (OECD, 2001, cited by NICNAS; 2013).

6. Discussion and conclusion

Citrates are naturally occurring ions, that are present in food, and are key in the basic physiological citric acid cycle (Krebs cycle). A recent, published authoritative review on the toxicity of citrates / citric acid is not available (EFSA re-evaluation in progress, as of May 2020). The latest available assessment appears to be that on citric acid by NICNAS (2013), which relies on an OECD SIAP (2001).

So far, EFSA (2016), in evaluating the acceptability of citrates as food additive had emphasised "*that citrate has a well-established role as an intermediate metabolite in the citric acid cycle and as a natural component of food*" and confirmed the 'ADI not specified' established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1974."

It is suggested to update this document if/when the ongoing EFSA re-evaluation on citric acid and citrates becomes available.

Citrate "in a nutshell":

Key / leading adverse systemic effect on human health:	Citrates are not considered to cause relevant systemic toxicological effects. Authoritative bodies have emphasised their well-established role as an intermediate metabolite in the citric acid cycle and as a natural component of food and have not established toxicological thresholds such as ADIs or DNELs.
Relevant (CLP) Hazard Classification(s) for systemic effects:	none
Numerical toxicological descriptor:	n/a

References

EFSA (2016): Safety of trimagnesium dicitrate anhydrous (TMDC) to be used as a food additive in food supplements in solid and chewable forms. EFSA Journal 2016;14(11):4599 doi: 10.2903/j.efsa.2016.4599

JECFA (1973/1974): Toxicological evaluation of some food additives including anticaking agents, antimicrobials, antioxidants, emulsifiers and thickening agents. WHO food additive series No.5 (evaluation agreed in 1973, published in 1974). Available online: http://www.inchem.org/documents/jecfa/jecmono/v05je24.htm (accessed 2020-05-25)

NICNAS (2013): Citric acid: Human health tier II assessment, 28 June 2013. https://www.nicnas.gov.au/chemical-information/imap-assessments/imap-group-assessment-report?assessment_id=198 (accessed 2020-05-25)

OECD (2001): SIDS Initial Assessment Report: Citric Acid. 11th SIAM, Orlando, Florida, USA, January 2001. https://hpvchemicals.oecd.org/ui/handler.axd?id=ff78c453-36c1-430d-9034-63e15899d24b (accessed 2020-05-25)

US FDA (1977): Evaluation of the Health Aspects of Citric Acid, Sodium Citrate, Potassium Citrate, Calcium Citrate, Ammonium Citrate, Triethyl Citrate, Isopropyl Citrate, and Stearyl Citrate as Food Ingredients. Report no. FDA/BF-78/96, Accession Number in National Technical Reports Library: PB280954. https://ntrl.ntis.gov/NTRL/dashboard/searchResults.xhtml?searchQuery=PB280954# (accessed 2020-05-25).