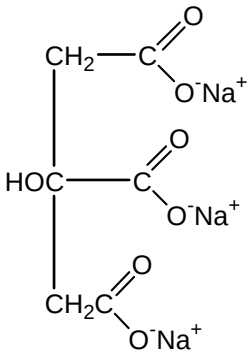
SODIUM CITRATE

Anhydrous (tri sodium citrate) form or Dihydrate form

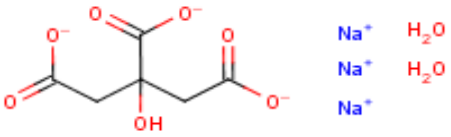
SYNONYMS

Citratin
Citric acid, trisodium salt
Citrosodine
Trisodium citrate
Trisodium citrate dihydrate

CHEMICAL STRUCTURE



Tri sodium citrate



Sodium citrate dihydrate

CHEMICAL FORMULA

$C_6H_5Na_3O_7$ **$C_6H_5Na_3O_7.3H_2O$**

IDENTIFIER DETAILS

CAS Number	:	68-04-2 (tri sodium citrate); 6132-04-3 (dihydrate)
CoE Number	:	-
FEMA	:	3026
EINECS Number	:	200-675-3
E Number	:	E331

CLP CLASSIFICATION

Ingredient CLP Classification: No

Endpoint	Classification	Category
Acute Oral Toxicity	-	-
Acute Dermal Toxicity	-	-
Acute Inhalation Toxicity	-	-
Skin Corrosive/irritant	-	-
Eye Damage/Irritation	-	-
Respiratory Sensitisation	-	-
Skin Sensitisation	-	-
Mutagenicity/Genotoxicity	-	-
Carcinogenicity	-	-
Reproductive Toxicity	-	-
Specific Target Organ Toxicity	-	-
Aspiration Toxicity	-	-

SPECIFICATIONS

Melting Point: >300^{o}C

Boiling point: Decomposes

PURPOSE

Flavouring compound

STATUS IN FOOD AND DRUG LAWS

CoE limits:

Beverages (mg/kg)	Food (mg/kg)	Exceptions (mg/kg)
-	-	-

Acceptable Daily Intake:

ADI (mg/kg)	ADI Set by	Date Set	Comments
NOT LIMITED	JECFA	1973	

FDA Status:(CFR21)

Section Number	Comments
184.1751, 582.1751 582.6751.	Direct food substance generally recognised as safe.

HUMAN EXPOSURE

Natural Occurrence: Citric acid is an intermediary substance in oxidative metabolism, being engaged in the tricarboxylic acid cycle. Citric acid and

citrates occur in many foods and are normal metabolites in the body (Gruber et al, 1948; Boweman *et al.,* 1990).

Citric acid and its salts occur naturally in many plants and animals. Sodium citrate is odourless and has a pleasant, acid taste. Sodium citrate can be anhydrous or it can contain two molecules of water of crystallization (Fenaroli, 2005).

Reported Uses: Sodium citrate is reportedly found (maximum levels) in baked goods 0.50 ppm, breakfast cereals 1.57 ppm, other grains 0.67 ppm, fats and oils 7.94 ppm, milk products 0.71 ppm, cheese 25.30 ppm, frozen dairy 0.66 ppm, fruit juice 4.32 ppm, fruit ices 2.0 ppm, meat products 0.22 ppm, processed vegetables 0.56 ppm, soft candy 2.32 ppm, jams and jellies 0.76 ppm, sweet sauce 1.07 ppm, gelatins & puddings 1.86 ppm, soups 0.10 ppm, snack foods 7.46 ppm, non-alcoholic beverages 0.80 ppm, alcoholic beverages 1.26 ppm, gravies 3.32 ppm, imitation dairy 1.10 ppm, hard candy 0.06 ppm, and instant coffee and tea 5.64 ppm (Fenaroli, 2005).

Sources other than foods: Sodium Citrate was first used as an anticoagulant in 1914 when it was used to prevent coagulation in blood products. Citrate, the active component of sodium citrate exerts its anticoagulant effects through chelating free ionised calcium, a necessary agent for coagulation. It is also used as an expectorant and systemic alkaliser. Saline expectorants are especially useful when they are required to liquefy thick, tenacious sputum. In the body, sodium citrate is oxidised to bicarbonate and excreted in the urine; thus, when given orally it is useful in acidosis and to overcome excessive urinary acidity (Osol *et al.,* 1975).

Sodium citrate also increases the rate of urinary excretion of calcium. Therefore, it has been employed in the treatment of hypercalcemia and to facilitate elimination of lead in poisoning. Sodium citrate is also used to prevent darkening when iron is added to preparations containing tannin (Osol *et al.,* 1975).

TOXICITY DATA

In Vivo Toxicity Status

Species	Test Type	Route	Reported Dosage
Rat	LD_{50}	Oral	6.5-12.1g/kg (Yokotani et al., 1971; Zang et al., 1987)
Mouse	LD_{50}	Oral	7.1g/kg (Yokotani et al., 1971)
Mouse	LD_{50}	Intravenous	42mg/kg
Mouse	LD_{50}	Intraperitoneal	961mg/kg
Rat	LD_{50}	Intraperitoneal	884mg/kg

(Gruber et al., 1948)

Carcinogenicity and Mutagenicity

Horn *et al.,* (1957) conducted a limited lifetime study with 20 male rats receiving up to 5% citric acid in the diet for up to 2 years, they reported no evidence of carcinogenicity (Horn *et al.,* 1957).

Dietary administration of Sodium citrate at 5% of the diet for 32 weeks to male F344 rats lead to an increase in the number of preneoplastic and neoplastic tumours in the bladder of rats exposed to a known bladder carcinogen. This increased incidence occurred with a change in a range of urinary parameters (increased pH, sodium ion concentration, increase in crystalline $MgNH_4PO_4$, Fukushima et al 1986). Sodium citrate has been reported to increase the levels of bladder DNA synthesis in the rat (Shibata *et al.,* 1992).

The co-administration of both 5% sodium citrate in the diet and two known bladder carcinogens (Administered in the drinking water) led to an increased incidence of bladder carcinomas. This was reportedly due to both the increased water consumption of this group and the increased urinary excretion of the carcinogen metabolites (Inoue *et al.,* 1988). In a study F334 rats were given a N-butyl-N-(4-hydroxybutyl) nitrosamine (a known bladder carcinogen) for 4 weeks, and then fed a diet containing 5% sodium citrate (approximately 1.9 g/kg/day) between weeks 4-8 and 11-20. One treatment group were administered uracil (an RNA component, and used to accelerate tumor promotion) at 3% of the diet between weeks 9-11. The incidence of bladder papillomas (benign tumors) was increased for the sodium citrate group compared to the group treated with both uracil and the bladder carcinogen. No papillomas developed in the group treated with uracil and sodium citrate (De Camargo *et al.,* 1991).

When the sodium citrate content of the diet fed to F344 rats was 1.7% (approximately 0.74 g/kg/day), following the same protocol of de Camargo *et al.,* (1991) There was no increase in the incidence of bladder tumours, when administered with uracil and a bladder carcinogen (Ono *et al.,* 1992). However, co-administration of both sodium citrate at 1.7% in the diet with sodium bicarbonate and sodium ascorbate lead to a synergistic increase in the incidence of carcinomas and papillomas of the bladder (Ono *et al.,* 1992). Ono *et al.,* (1992) also reported that there was no increased incidence of DNA synthesis in the bladder of rats exposed to sodium citrate at 1.7% of the diet.

Dermal toxicity

Citric acid has been reported to be mildly irritating when applied to the skin of rabbits for 24 hours (Marhold, 1986). In humans skin testing of 60 eczema patients with 2.5% citrate acid in petrolatum was found to be non-irritating (Niinimäk,i 1987). However, an irritant skin dermatitis due to citric acid has been reported amongst bakers and waiters (exposure levels were not specified, Fisher 1986).

Severe eye damage was reported in a patient who had splashed a saturated citric acid solution in to his eye (Villiard 1927). Severe and permanent eye damage occurred in rabbit eyes washed for 30 minutes in citric acid solutions (Carpenter *et al.,* 1946). Application of 750 μg of Citric acid to the eye for 24 hours was reported to cause severe eye injury to rabbits (Grant 1986). However, sodium citrate unlike citric acid is reported to be well tolerated by the eye, with 10% eye drops being used to treat chemical burns of the eye (Grant 1986).

Reproduction and Developmental toxicity

No reproductive effects were reported in limited studies in which rats received diets containing 1.2% citric acid and 0.1% sodium citrate for 29 weeks prior to mating and then a few months after mating had occurred (Bonting *et al.,* 1956). No reproductive effects were reported in other studies in which rats received 5% citric acid (approximately 2.5 g/kg/day) prior to, during and subsequently after mating (Wright *et al.,* 1976).

In mice receiving 5% citric acid (approximating to 7.5 g/kg/day), in the diet prior to, during and after mating, there was no effect of treatment upon any reproductive parameters or any evidence of teratogenicity (Wright *et al.,* 1976). There was also no evidence of teratogenicity or any reproductive effects on female hamsters exposed to 272 mg/kg/day on days 6-10 of pregnancy or pregnant white rabbits exposed up to 425 mg/kg/day of citric acid (by an unspecified route, FDRL 1973).

No teratogenic effects were observed in single-comb white leghorn chick embryos after sodium citrate (dissolved in water) 10.00 mg/egg was injected into the air cell after 96 hr incubation (Verret *et al.,* 1990)

Inhalation toxicity

Apparently, involuntary coughing was induced in humans exposed to aerosols for a few seconds containing between 2.5-32% citric acid (Pounsford *et al.,* 1985). Schreiber *et al.,* (1986) reported that in 23 human male volunteers, involuntary coughing occurred when they were exposed to citric acid at concentrations between 0.5-32 mg/ml. Citric acid (at an unspecified concentration) has been reported to induce bronchoconstriction in human asthmatics (Lindeman *et al.,* 1989).

On exposing guinea pigs to citric acid at atmospheric concentrations of either 31.1 or 81 mg/m^3, significant coughing was reported to occur in the top dose only (Zelenak *et al.,* 1982). Coughing was also reported in guinea pigs exposed to 75 mg/ml of citric acid as an aerosol for 3 minutes with bronchoconstriction occurring after 3-4 minutes (Forsberg *et al.,* 1986).

The addition of sodium citrate at 12 ppm to reference cigarettes, used in a 90 day-sub-chronic inhalation exposure in rats, led to a series of pathological changes to smoke exposure that were indistinguishable from those changes caused by the control cigarettes. This indicated that addition of sodium citrate

to a reference cigarette had no discernable effect upon the type or severity of the treatment related pathological changes associated with tobacco smoke exposure [Baker *et al.,* 2004]

When tested at 0.8 ppm in cigarettes, in a 13-week inhalation study, the presence of sodium citrate "…had no discernible effect on the character of extent of the biologic responses normally associated with inhalation of mainstream cigarette smoke in rats."(Gaworski *et al.*, 1998).
However, it should be noted that the cigarettes had been spiked with a number of flavour ingredients in combination prior to smoking, and they contained a typical American blend humectant and sugar component (*i.e.* glycerine ≈ 20,000 ppm, propylene glycol at ≈ 24,000 ppm, and brown invert sugar at ≈ 24,000 ppm).

Other relevant studies

The citrate moiety is reported to be rapidly metabolised in the tricarboxylic acid cycle to CO_2 and water leaving 3 mols of Na^+ for each mol of sodium citrate ingested. Sodium citrate is metabolised by the liver, skeletal muscle and renal cortex. As a result, patients with impaired liver or renal systems are at increased risk to develop symptoms of citrate toxicity. A natural tolerance to citrate is primarily determined by an individuals body weight, blood volume and hematocrit (Boweman *et al.,* 1990).

Ingestion of citric acid frequently or in large doses may cause erosion of teeth and local irritation, apparently because of the low pH: the effects also occur with lemon juice which contains about 7% of citric acid and has a pH of less than three. A 1% solution has been used as a cooling drink in fever. Potassium citrate has been used in daily doses totalling up to 10 g as a mild diuretic, to render the urine less acid and as a potassium supplement. Sodium citrate has also been used in daily doses of up to 10 g to render the urine less acid and as a mild diuretic (Martindale, 1972).

A single intrapulmonary injection of 3.8% trisodium citrate and acid citrate dextrose, in to rabbits has been reported to cause extensive degeneration and necrosis of alveolar pnemocytes including the type II pneumocyte and bronchiolar or bronchial epithelial cells Subsequently the alveoli and alveolar ducts collapsed and leading to the proliferation of fibroblasts. (Mitsuhashi *et al.,* 1985). It was suggested by the authors that this may be a useful animal model for pulmonary fibrosis.

Potassium and sodium citrate at oral doses of up to 4 g per person have been extensively used in medical practice for many years without giving rise to ill effects. The calcium salt (calcium citrate) is reported to probably behave similarly in the body. As food additives, therefore, all the citrates can be considered as one group. There is no reason to believe that the use of these citrates as food additives constitutes a significant toxicological hazard to man (JECFA 1974).

A young woman who was reported to have ingested almost 25 g of citric acid, vomited and almost died (Nazario, 1952). Transfusions of large volumes of citrated blood have also been reported to cause hypocalcaemia (a depletion of body calcium), which may lead to nausea, increased muscle weakness, breathing difficulties and even cardiac arrest (Boweman *et al.,* 1990).

Numerous studies have used exogenous administration of sodium bicarbonate (NaHCO(3)) and sodium citrate (Na-citrate) in an attempt to enhance human performance (Requena *et al.,* 2005). After ingestion of NaHCO(3) and Na-citrate, two observations were reported to have been made: (a) There was great individual variability in the ergogenic benefit reached, which was attributed to the level of physical conditioning of the subjects and to their tolerance of the buffer substance; and (b) the subjects who had ingested NaHCO(3) and Na-citrate showed higher levels of pH, bicarbonate, and lactate ions concentrations in their exercising blood than do the subjects who had ingested the placebo. A majority of the studies have suggested that the ingestion of both substances provides an ergogenic effect due to the establishment and maintenance of an elevated pH level during exercise. However, Requena et al., (2005) report that the exact mechanism by which the ergogenic effects occur has not been conclusively demonstrated. Sodium bicarbonate and Na-citrate seem to be effective in activities with a sufficient duration to generate a difference in the hydrogen ion gradient, characterized by a very high intensity and involving large muscular groups. However, in activities of equally high intensity, but with longer duration, the results obtained have been conflicting and inconclusive [Requena *et al.,* 2005].

Administration of aluminium to Swiss Webster mice at 3 or 1000 micrograms in the diet, which also contained 3.5% sodium citrate, for 7 weeks. The feeding of both aluminium (Al) and citrate to mice lead to the accumulation of Al in the central nervous system and this was associated with overt signs of neurotoxicity (Oteiza *et al.,* 1993).

Twenty male rats receiving 5% sodium citrate for 32 weeks (about 2.5 g/kg /day) apparently showed no signs of toxicity (Fukushima *et al.,* 1986). Examination of the urine of rats receiving 5% sodium citrate for 16 weeks, the rats had an increased urinary pH, crystal content and sodium concentration (Fukushima *et al.,* 1986). Conflictingly in the mouse, a decreased mean survival, life time (11-13 months as opposed to 16-17 months in control mice) and growth rate was reported in mice receiving citric acid at 5% in the diet (Wright *et al.,* 1975).

Diets fed to rats containing 1.2% of citric acid had no harmful effect on the growth of two successive generations of rats over a period of 90 weeks. No detrimental effect could be observed on reproduction. No significant changes were noted in the haematology or biochemistry nor was there any other pathological finding that could be attributed to the treatment. Loss of calcium or other fixed bases was not observed. The dental attrition was found to be slightly more marked than in the control groups (Bonting *et al.,* 1956).

In a 2 year rat study (20 rats per sex per group), with dose levels of 5% and 3% citric acid in the diet, the only finding was decreased food consumption. There were no macroscopic or microscopic findings related to treatment (Horn *et al.,* 1957). However in a study conducted by Yokotani *et al.,* (1971) noted that in groups of 10 male rats fed 0, 1.2, 2.4 or 4.8 % citric acid in the diet for 6 weeks, decreased food consumption was noted for all treated groups. The top dose group also had mildly altered blood and urine parameters and slight degeneration of the spleen and thymus gland (Yokotani *et al.,* 1971).

The only effect reported in groups of guinea pigs receiving diet supplemented with 1-5% citric acid was a reduced haematological parameter, packed cell volume. No macro or micropathology was conducted (Wright *et al.,* 1975). No adverse effects were reported in limited studies in 15 rabbits fed sodium citrate at 7.7 % in the diet for 150 days (Packman *et al.,* 1963).

Progression of autosomal-dominant polycystic kidney disease (ADPKD) in the heterozygous male Han:SPRD rat is dramatically slowed by ingestion of potassium or sodium citrate. This study examined the efficacy of delayed therapy with sodium citrate, the effect of sodium citrate therapy on kidney cortex levels of transforming growth factor-beta (TGF-beta), and the response to calcium citrate ingestion. Rats were provided with citrate salts in their food, and renal clearance, blood pressure, blood chemistry, and survival determinations were made. Sodium citrate therapy was most effective when started at age 1 month, and delay of therapy until age 3 months produced no benefit. Kidney cortex TGF-beta levels were elevated in 3- and 8-month-old rats with ADPKD, but not in 6-week-old rats. Sodium citrate treatment, started at age 1 month, lowered TGF-beta levels to normal in 3-month-old rats, but this is probably not the primary mechanism of citrate's beneficial effect. Calcium citrate had only a modest effect in preserving glomerular filtration rate. Effective treatment of ADPKD in this rat model requires early administration of a readily absorbed alkalinizing citrate salt. Existing data on ADPKD patients on vegetarian diets or with kidney stones should be studied in light of these findings (Tanner & Tanner 2003).

The pharmacokinetics and metabolism of sodium citrate was investigated in a prospective cohort study in serial arterial blood samples in cirrhoticcritically ill and noncirrhotic patients (16 of each) after infusion of sodium citrate (0.5mmol.kg.hr-1) and calcium chloride (0.17 mmol.kg-1.hr-10) for two hours. The clearance rate (total body clearance) of citrate was significantly reduced in critically ill cirrhotic patients (710mL/min) compared to non cirrhotic critically ill patients (340mL/min). Citrate peak concentrations over time were also increased by 65 and 114% in cirrhotic patients (similar volumes of distribution). No citrate related side effects or significant metabolic changes were observed. It was concluded by the author that hepatic citrate metabolism effects citrate clearance in cirrhotic patients and when coupled with the pharmacokinetic data may provide a basis for the clinical use of citrate anticoagulation in critically ill patients (Kramer *et al.,* 2003).

Tri-sodium citrate has been used as an anticoagulant in patients with heparin intolerance. Good initial tolerance of the compound was observed. However,

after a few weeks (exact time not stated) alkalosis developed in all the patients and the pre-dialysis bicarbonate levels rose from 27nmol/l to 40mmol/l. A progressive rise in pre-dialysis sodium levels 136mmol/l to 150mmol/l and cellular aluminium levels from 3microg/l to 38microg/l also occurred. The later increase in aluminium was controlled by replacing the glass bottles with polyvinylchloride bags (with a negligible aluminium content) (De Vos & Hombrouckx, 2003).

In evaluating the acceptance of citric acid, JECFA (1974) concluded that whilst emphasis was placed on its well-established metabolic pathways. Toxicological studies on animals supplement this information. Citric acid and its calcium, potassium and sodium salts do not constitute a significant toxicological hazard to man, (JECFA, 1974).

Oopik et al., (2010) conducted a study to determine if the ingestion of sodium citrate altered blood levels of fluid and electrolyte regulatory hormones at rest and during exercise. They conducted a randomized, double-blinded, crossover design trial in which 13 young well trained male runners performed continuous incremental running tests to exhaustion, 2 h after ingestion of 0.5 g/kg body mass of sodium citrate or a placebo in 1000 ml of solution. The trials were separated by two weeks. Aldosterone is a hormone that increases the re-absorption of sodium and water and the release of potassium in the kidneys – this increases blood volume and therefore increases blood pressure. Aldosterone concentration did not differ between the two trials before ingestion of test material. However, pre-exercise ingestion of sodium citrate induced a decrease in serum aldosterone concentration both in the resting condition and during incremental running exercises. The authors deliberate that the observed effect of sodium citrate on the serum aldosterone level may be mediated by an acute increase in plasma volume and serum sodium concentration alterations, [Oopik *et al*., 2010].

Bell *et al*., (2007) reports a case of severe citrate toxicity during volunteer donor apheresis platelet collection. The donor was a 40-year-old female, first-time apheresis platelet donor. Past medical history was remarkable for hypertension, hyperlipidemia, and depression. Reported medications included bumetanide, pravastatin, and paroxetine. Thirty minutes from the start of the procedure, the donor noted tingling around the mouth, hands, and feet. She then very rapidly developed acute onset of severe facial and extremity tetany. Empirical treatment with intravenous calcium gluconate was initiated, and muscle contractions slowly subsided over approximately 10 to 15 minutes. The events are consistent with a severe reaction to calcium chelation by sodium citrate anticoagulant resulting in symptomatic systemic hypocalcemia. Upon additional retrospective analysis, it was noted that bumetanide is a loop diuretic that may cause significant hypocalcemia. We conclude that careful screening for medications and underlying conditions predisposing to hypocalcemia is recommended to help prevent severe reactions due to citrate toxicity. Laboratory measurement of pre-procedure serum calcium levels in selected donors may identify cases requiring heightened vigilance. The case also illustrates the importance of maintaining preparedness for managing rare but serious reactions in volunteer apheresis blood donors, [Bell *et al*., 2007].

Behavioural data

No data identified.

In Vitro Toxicity Status

Carcinogenicity and mutagenicity

Additional information concerning the *in vitro* mutagenicity of this material may be found in “An Interim report on data originating from Imperial Tobacco Limited’s Genotoxicity testing programme September 2003” or “An updated report on data originating from Imperial Tobacco Limited’s external Genotoxicity testing programme – Round 2 August 2007”.

Sodium citrate was found to be negative in the Ames *Salmonella typhimurium* in the following strains TA 97 and TA102 at 0.1-10 mg/plate, both with and without metabolic activation (Fujita *et al.,* 1992).

Sodium citrate did not induce chromosomal damage in mammalian hamster cells (Ishidate *et al.,* 1984).

Similarly it was not mutagenic in *Escherichia coli* or *Saccharomyces cerevisiae*, either with or without a liver metabolic activation system (Hayes *et al.,* 1984).

Baker *et al.,* [2004]; examined the effects of the addition of 482 tobacco ingredients upon the biological activity and chemistry of mainstream smoke. The ingredients, essentially different groups of flavourings and casings, were added in different combinations to reference cigarettes. The addition of sodium citrate at 12 ppm was determined not to have affected the mutagenicity of the total particulate matter (TPM) of the smoke in either the Ames, *in vitro* micronucleus assay or the neutral red assay when compared with that of the control cigarettes [Baker *et al.,* 2004].

The mutagenicity of the smoke condensate was assayed in the *Salmonella* plate incorporation [Ames] assay with the tester strain TA98 in the presence of an S9 metabolic activation system. The cytotoxicity of the cigarette condensate was determined in the neutral red uptake assay and the (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H tetrazolium, inner salt assay (MTS assay) with the human hepatocellular liver carcinoma cell line, HEP-G2. It was concluded that the *in vitro* mutagenicity and cytotoxicity of the cigarette smoke was not increased by the addition of the ingredients, which included *sodium citrate* at levels up to 61 ppm.

Other relevant studies

Yousefi *et al.,* (2004) demonstrated cell membrane destruction activity by carboxylic acid derivatives (CADs) mainly tri-sodium citrate, in neoplastic cell lines and, to a far lesser extent, in normal human peripheral blood

mononuclear cells (hPBMC). Flow cytometric (FACS) analysis was applied to Annexin-V and Propidium Iodide (PI) stained cells to evaluate the degree of the apoptosis induced by citrate in the following cell lines: CCRF-CEM (shortened to CEM), H9, and Jurkat (T-Cells), Raji and WIL2-NS (B-Cells), HL-60 (myeloblasts), K562 (myelocytes) and U937 (monocytes). They also tested normal hPBMC. Before staining with Annexin/PI, manual cell counts were performed on 24- and 48-h-old cell cultures. Cell supernatants were assayed for lactate dehydrogenase (LDH). LDH values in samples correlated with enhanced apoptosis by FACS analysis. In addition, the ability of tri-sodium citrate to induce apoptosis in the presence and the absence of several antineoplastic drugs, such as dexamethasone, arsenic trioxide, hydrocortisone, 6-mercaptopurine, and methotrexate were tested on Jurkat cells. FACS, LDH, and cell count values all demonstrated an enhanced degree of apoptotic cell death in Jurkat cells by citrate. In most of the investigated cell lines, except for the H9 cell line, citrate induced a greater degree of apoptosis than acetate which induced a greater degree than lactate. Yousefi *et al.,* (2004) reported the cell death by ascorbate appeared to be due to necrosis rather than apoptosis. The authors suggested that citrate might be of benefit in some chemotherapy treatments in order to reduce drug toxicity or possibly enhance drug activities in certain neoplasias [Yousefi *et al.,* 2004].

PYROLYSIS AND TRANSFER STUDIES

Information relating to the pyrolysis and/or transfer of Sodium Citrate is detailed in the Report on Thermochemical Properties of Ingredients document. In the aforementioned document, the term ‘pyrolysis’ means the heating of an ingredient in isolation under controlled conditions in an analytical device to examine its degradation potential. The expression ‘transfer data’ on the other hand is used to describe the fate of an ingredient in qualitative and quantitative terms following the smoking of a tobacco product to which it has been applied.

In an extension to the Baker and Bishop (2004) study, a further 159 ingredients were analysed. Under pyrolysis Sodium Citrate’s pyrolysate contained Methylisobenzofuranone (13.9%), 2-Butanone (5.4%), Methylcyclopentenone (5.2%), Methylbenzofuraone (4.6%), Methylfuranone (4.3%), Cresol (1.9%), Toluene (1.8%), and Styrene (1.1%), [Baker and Bishop, 2005].

REACH Statement

This ingredient has been registered under REACH. Under REACH, registrants have an obligation to provide information on substances they manufacture or import. This information includes data on hazardous properties (covering various toxicological endpoints), guidance on safe use and classification and labelling. The European Chemicals Agency (ECHA) makes this information publicly available on its website: http://echa.europa.eu/.

REFERENCES

Baker RR, *et al.,* (2004) An overview of the effects of tobacco ingredients on smoke chemistry and toxicity. *Food Chem Toxicol*. **42** Suppl: S53-83.

Baker and Bishop, (2005). The pyrolysis of non-volatile tobacco ingredients using a system that simulates cigarette combustion conditions. *J. Anal. Appl. Pyrolysis.* **74**: 145-170.

Bell *et al*., (2007). Severe citrate toxicity complicating volunteer apheresis platelet donation. *J Clin Apher.* **22(1):** 15-6.

BIBRA (1993) Toxicity profile: Citric acid and its common salts.

Bonting*, et al.,* (1956) *Voeding* **17**: 137.

Boweman *et al.,* (1990) In Textbook of Pharmacology. 2nd Edition Blackwell Scientific Publications London.

Carpenter *et al.,* (1946) *American Journal of Ophthalmology* **29**: 1363 (As cited in Grant 1986).

De Camargo *et al.,* (1991) Summation effects of uracil and other promoters on epithelial lesion development in the F344 rat urinary bladder initiated by N-butyl-N-(4-hydroxybutyl) nitrosamine. *Japanese Journal of Cancer Research*. **82(11):** 1220-1225.

FDRL (1973) Food and Drug Research Laboratories Inc. Teratologic evaluation of FDA 71-54 (Citric acid) in mice and rats, hamsters and rabbits. Contract number FDA 71-260 (As cited in BIBRA 1993).

Fenaroli (1995) Fenaroli's Handbook of Flavor Ingredients, Volume II 3rd Edition. pp 278.

Fenaroli's Handbook of Flavor Ingredients (2005). Fifth Edition. CRC Press. ISBN: 0-8493-3034-3.

Fisher (1986) Contact Dermatitis 3rd Edition Lea and Febiger, Philadelphia. p 420. (As cited in BIBRA 1993).

Forsberg *et al.,* (1986) Cough induced by stimulation of capsaicin –sensitive sensory neurones in conscious guinea -pigs. *Acta Physiology Scandinavian* **128(2):** 319-320.

Fujita *et al.,* (1992) Mutagenicity test of food additives with *Salmonella typhimurium* TA97 and TA102 VII *Kenkyu Nenpo- Toyko-Toristu Eisei Kenkyusho* **43:** 219-227.

Fukushima *et al.,* (1986) Sodium citrate: a promoter of bladder carcinogenesis. *Japanese Journal of Cancer Research*. **77(1):** 1-4.

Gaworski *et al.*, (1998). Toxicologic evaluation of flavor ingredients added to cigarette tobacco: 13-week inhalation exposure in rats. *Inhalation Toxicology*. **10**: 357-381.

Grant (1986) Toxicology of the eye, 3rd edition. Charles C. Thomas. Springfield Illinois.

Gruber, *et al.,* (1948) *Journal of Pharmacology & Experimental Therapy*. **94:** 65.

Hayes *et al,* (1984) *Mutation Research*. **130**: 97.

Horn *et al.,* (1957) *Journal of Agricultural Food Chemistry.* **5(10)**: 759.

Inoue *et al.,* (1988) combined effects of L-ascorbic acid, citric acid or their sodium salts on tumor induction by N-butyl-N-(4-hydroxybutyl) nitrosamine or N-ethyl-N-(4-hydroxybutyl) nitrosamine in the rat urinary bladder. *Cancer Letters.* **40(3):** 265-273.

In vitro toxicity testing of tobacco ingredients in burnt form (Internal document R-030).

Ishidate *et al.,* (1984)Primary mutagenicity screenig of food additives currently used in Japan. *Food & Chemical Toxicology*. **22(8)** 623-636.

Kramer L, Bauer E, Joukhadar C, Strobl W, Gendo A, Madl C & Gangl A (2003). Citrate pharmacokinetics and metabolism in cirrhotic and noncirrhotic critically ill patients. *Critical Care medicine.* 31(10): 2450-2455.

Lindeman *et al.,* (1989) Effect of magnesium sulphate on bronchoconstriction in the lung periphery. *Journal of Applied Physiology*. **66(6):** 2527-2532.

Martindale's Extra Pharmacopoeia, 26th Ed. (1972) The Pharmaceutical Press, London.

Mitsuhashi *et al.,* (1985) Experimental pulmonary fibrosis induced by trisodium citrate and acid- citrate-dextrose. *Experimental & Molecular Pathology*. **42(2):** 261-270.

Niinimaki *et al.,* (1987) Scratch chamber tests in food handler dermatitis. *Contact Dermatitis* **16(1):** 11-20.

Ono *et al.,* (1992) Synergism of environmental carcinogens and promoters on bladder cancer development initiated by N-butyl-N-(4-hydroxybutyl) nitrosamine in F344 rats. *Japanese Journal of Cancer Research*. **83(9):** 955-963.

Oopik V, Timpmann S, Hackney A C, Kadak K, Medijainen L, Karelson K (2010). Ingestion of sodium citrate suppresses aldosterone level in blood at rest and during exercise. *Appl Physiol Nutr Metab* **35** (3): 278-285

Osol *et al.,* (1975). Remington's Pharmaceutical Sciences. 15th ed. Easton, Pennsylvania: Mack Publishing Co.pp. 802.

Oteiza *et al.,* (1993) Aluminium accumulation and neurotoxicity in Swiss-Webster mice after long-term dietary exposure to aluminium and citrate. *Metabolism* **42(10):** 1296-1300.

Packman *et al.,* (1963) *Toxicology & Applied Pharmacology*. **5:** 163.

Pounsford *et al.,* (1985)Effect of bronchodilators on the cough response to inhaled citiric acid in normal and asthmatic subjects. *Thorax* **40(9):** 662-667.

Requena B *et al.,* (2005). Sodium bicarbonate and sodium citrate: ergogenic aids? *J Strength Cond Res*. **19(1):** 213-24.

Tanner GA, Tanner JA (2003). Dietary citrate treatment of polycystic kidney disease in rats. *Nephron Physiology*. **93**(1): P14-20

Verrett *et al.* (1980). Toxicity and teratogenicity of food additive chemicals in the developing chicken embryo. *Toxicology & Applied Pharmacology.* **56**: 265.

Villiard (1927) *Archives of Ophthalmology* (Paris) **44**: 222 (As cited in Grant 1986).

Werken Glorieux AZ & Hombrouckx R (2003). Citrate anticoagulation and adverse effects. *Journal EDTNA ERCA*. **29(3):** 112-113, 117.

Wright (1975) *Nutrition (London)* **29:** 367.

Wright (1976 b) *Nutrition Reports*. 13(6): 563.
Wright (1976a) Some effects of dietary citric acid in small animals. *Food & Cosmetic Toxicology* **14:** 561.

Yokotani *et al.,* (1971) *Journal Takeda Research Laboratory*. **30(1):** 25.

Yousefi S *et al.,* (2004) Citrate shows specific, dose-dependent lympholytic activity in neoplastic cell lines**.** *Leuk Lymphoma*. **45(8):**1657-65.

Zang *et al., Acta Acad Med. Shanghai* **14:** 195 (As cited in BIBRA 1993).

Zelenak *et al.,* (1982) Assessment of the cough reflex caused by inhalation of sodium lauryl sulphate and citric acid aerosols. *Fundamentals of Applied Toxicology.* **2(4):** 177-180.