Annex I to the CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2

International Chemical Identification: Citric acid

EC Number: **201-069-1**

CAS Number: **77-92-9**

Index Number: **N/A**

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1 PHYSICAL HAZARDS

1.1 Explosives

1.1.1 Tremain S (2006)

Study reference:

Tremain S. (2006), Determination of Hazardous Physico-Chemical Properties, Safepharm Laboratories Study No 2189/003

Detailed study summary and results:

Please refer to Section A3 of the CAR (Attachment 1).

1.2 Flammable gases (including chemically unstable gases)

Not applicable.

1.3 Oxidising gases

Not applicable.

1.4 Gases under pressure

Not applicable.

1.5 Flammable liquid

Not applicable.

1.6 Flammable solids

1.6.1 Tremain S (2006)

Study reference:

Tremain S. (2006), Determination of Hazardous Physico-Chemical Properties, Safepharm Laboratories Study No 2189/003

Detailed study summary and results:

Please refer to Section A3 of the CAR (Attachment 1).

1.7 Self-reactive substances

No study available.

1.8 Pyrophoric liquids

Not applicable.

1.9 Pyrophoric solid

No study available.

1.10 Self-heating substances

No study available.

1.11 Substances which in contact with water emit flammable gases

No study available

1.12 Oxidising liquids

Not applicable.

1.13 Oxidising solids

1.13.1 Tremain S (2006)

Study reference:

Tremain S. (2006), Determination of Hazardous Physico-Chemical Properties, Safepharm Laboratories Study No 2189/003

Detailed study summary and results:

Please refer to Section A3 of the CAR (Attachment 1).

1.14 Organic peroxides

Not applicable.

1.15 Corrosive to metals

Not applicable.

2 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

2.1.1 Dickens F (1941)

Study reference:

Dickens F. (1941), The citric acid content of animal tissues, with reference to its occurrence in bone and tumour. Biochem. J. 35: 1011 – 1023

Detailed study summary and results:

Various tissues from several mammal species (including man, mouse, guinea-pig, rabbit, cat, dog, horse and oxen) were analysed for total citric acid content using an extraction and colorimetric method of analysis. Age, sex, source and strain of various test animals were not specified.

Test method

Freshly excised tissues (including hair, skin, liver, kidney, skeletal muscle, brain, testis, seminal vesicles, and bone including bone marrow) used in all experiments except horse hair and bone. Animals received last feed 18 hours before the experiment.

After dissection and weighing, tissues were ground with sand and extracted three times with 10 % trichloroacetic acid. When bone was analysed, it was first chopped or pulverised, then ground with sand and digested, usually with more concentrated (ca. 40 %) trichloroacetic acid at about 30 ºC. Any solid residue was re-extracted with 10 % trichloroacetic acid as before. After boiling down the combined extracts with sulphuric acid and cooling, the precipitated calcium sulphate was removed by centrifugation.

Citric acid was estimated using a colorimetric method. The original paper identifies sources of error considered to require special attention. Specific analytical techniques used to minimise the occurrence of error in the final method are stated.

Results: Distribution

Distribution in solid tissues discussed only.

A review of existing data up to the time of the paper's publication observed the following amounts of citric acid in the following body fluids:

Cerebrospinal fluid, amniotic and follicular fluids, blood serum, aqueous humour, blood, salvia and sweat contain in diminishing order 5 to 0.1 mg/100 ml

Semen contains up to 410 mg/100 ml associated with secretion of seminal vesicles which contain as much as 633 mg/100 ml.

Up to 1.6 % of dry, fat extracted bone consists of citric acid.

See Table 4.1.1(1)-1 for details of concentrations of citric acid found in the different tissues studied in each animal.

High concentrations of citric acid in fur and skin were attributed to concentrations in sebaceous glands.

Complete analysis of a mouse was undertaken to determine where the greatest concentration of the total citric acid in a mouse (10 mg/100 g) was situated. The mouse live weight was 31.2 g. Results are shown in Table 4.1.1(1)-2. Approximately 70 % of citric acid is in the skeleton.

Further experiments on ox bone, and skeletons of kittens (Age: 3 days) and puppies (age not stated) revealed that the greatest amounts of citric acid are found in the hard bone, rather than the bone marrow.

Conclusion

Citric acid is found naturally in all body tissues. In the skin, it is concentrated in sebaceous glands. The greatest distribution of citric acid in the body is found in the hard tissue of bones.

Table 1: Results of solid tissue investigations

Test species	Tissue	Citric acid concentration (mg/100 g)
Rabbit	Liver	2.8
	Skeletal muscle	2.5
	Kidney	6.0
	Whole brain	4.6
Guinea-pig	Liver	1.6
	Kidney	3.9
	Whole brain	3.8
	Testis	11.5
Mouse	Skin – fur removed	12.2
	Fur	133
	Seminal vesicle	128
	Skeleton plus eyes and connective tissue	46

	Muscle	2.8
	Skin fur and tail sheath	8.0
	Seminal vesicles, testes and prostate	110
	Viscera and brain	4.6
Horse	Hair	27.7
Man	Cyst	11.7

Table 2: Analysis of a mouse

Tissue	**Tissue weight (g)**	**Citric acid (mg)**	**% of total citric acid in mouse**
Skeleton with adherent connective tissue and eyes	8.2	3.75	69
Muscle with connective tissue and some fat	0.8	0.19	3.5
Outer skin including tail and fur	4.3	0.37	6.8
Seminal vesicles, testes and prostate	0.6	0.71	13.1
Liver, spleen, brain, heart, lungs, kidneys, and other viscera	9.0	0.41	7.5

2.1.2 Lussier JP (1957)

Study reference:

Lussier JP. (1957), Incorporation de l'acide citrique radioactive dans le squelette du rat blanc. Rev. Can. Biol. 16: 434-444

Detailed study summary and results:

Male Sprague-Dawley rats weighing 120 g were divided into two groups. One group were injected intraperitoneally with 25 units of parathyroid hormone two times per day for eight days. During this time, their weight increased to 150 g. The second group served as a control once their weight also reached 150 g. Rats in both groups were given an intraperitoneal injection of 2 mg of citric acid with a radioactivity of 1 million counts per minute immediately after having eaten. Exposure periods were 15 minutes to 24 hours with observations at 1, 5 and 8 hours of CO_2, urine and tissues. Rate of utilisation by whole organism and rate of incorporation into bone investigated.

Radioactivity was measured in respiratory carbon dioxide and urine. Residual radioactivity was considered to be distributed in tissues, mainly in bones.

Respiratory CO_2 accounts for 60 % of applied dose four hours after injection. After this time, no further radioactivity is found in respiratory CO_2.

Radioactivity found in urine is small during the first five hours after injection. However, 10 % is recovered at the end of 8 hours. It is assumed that not only citric acid but other metabolites are carrying the radioactivity.

Maximum incorporation of citric acid in bone occurs one hour after injection, up to 5 % of the dose is then recovered. Desorption rate from bone is rapid at the beginning but slows down afterwards. After 8 hours, 2 % remains. The radioactive CO_2 found in bone carbonate follows the same pattern, but the amount of radioactivity involved is much less.

Rats injected with parathyroid hormone show a 3 to 7-fold increase in the incorporation of radioactive citratein bone, all other measurements are within normal limits.

Conclusion

Citric acid is metabolised rapidly in rats and distributed into bone from where it is desorbed into metabolic systems.

Table 3: Distribution of radioactivity over exposure period

	% of applied dose		
	1 hour	5 hours	8 hours
Carbon dioxide (respiration)	25	60	60
Urine	1	2	10
Bone: carbonate	2	<1	< 0.5
Bone: citrate	5	2.5	2
Tissue (by difference)	67	35	27

2.1.3 Saffran M. Denstedt OF. (1951)

Study reference:

Saffran M., Denstedt O.F. (1951), The Effect of Intravenously Injected Citrate on the Serum Ionised Calcium in the Rabbit, Canadian Journal of Medical Sciences, Vol. 29, 245-254

Detailed study summary and results:

The study was undertaken to ascertain the rate at which calcium ions are replaced after having been removed by injected citrate and the capacity of the rabbit to cope with repeated doses of citrate.

Method

Test species: rabbit
Weight: Small dose – 3.0 kg, Toxic dose – 3.3 kg, Lethal dose – 3.5 kg
One rabbit / group
No control animals were used in the study. However, control samples were taken prior to injection of the test material.
Concentration of test substance: Small and toxic dose: 15 % solution of sodium citrate (dihydrate). Lethal dose: 30 % solution of sodium citrate.
Volume applied:
Small dose: 1 ml of a 15 % solution, injected twice, 5 minutes apart
Toxic dose: 2 ml of a 15 % solution
Lethal dose: 1.4 ml of a 30 % solution
Sampling time (Blood only):
Small dose: 7 minutes after second injection
Toxic dose: Immediately prior to injection and after the convulsions had ceased
Lethal dose: Immediately prior to injection and immediately after death

Small dose of citrate:

A control blood sample was taken from the ear vein of a 3.0 kg rabbit. 1 ml of a 15 % solution of sodium citrate was injected intravenously and after an interval of 5 minutes the dose was repeated. 7 minutes after the second injection, another blood sample was collected and the serum analysed for total calcium and for citric acid.

Toxic dose of sodium citrate:

A single rapid injection of 2 ml of a 15 % solution of sodium citrate was given by the ear vein to a 3.3 kg rabbit. During the experiment, blood samples were taken prior to the injection of citrate and after the convulsions ceased.

Lethal dose of sodium citrate:

1.4 ml of a 30 % solution of sodium citrate was injected rapidly into the ear vein of a 3.5 kg rabbit.

Clearance of citrate on repeated injection:

To determine the tolerance to repeated smaller doses, non-toxic amounts of sodium citrate solution were injected at half-hour intervals. A control blood sample was taken at the start and t 5 minutes before and after each injection.

Results

Effect of a small dose of citrate:

No physiological sign of toxicity was observed. The slow intravenous injection of 300 mg of sodium citrate did not cause significant reduction in the ionised calcium of the blood plasma.

Effect of a toxic dose of sodium citrate:

Immediately following the injection, the animal showed signs of acute hypocalcaemia and suffered a convulsive seizure. Within 5 minutes, however, it appeared to have made a complete recovery. It is evident that the restoration of the calcium ions of the serum after injection of citrate was very rapid and that the concentration was restored nearly to normal within a few minutes.

Effect of a lethal dose of sodium citrate:

Immediately following injection the animal showed signs of severe hypocalcaemia and died in convulsions within 5 minutes

Dosage and distribution

When citrate is administered the greatest portion of it is oxidised to carbon dioxide and water. Some may be converted to glycogen, another portion may be deposited as such in the tissues, particularly the bones, and still another portion may be excreted in the urine.

Metabolism

Citrate is metabolised by most tissues, notably kidney cortex, liver and muscle and it is stored in the bone. Also it is readily concentrated and excreted by the kidney.

Clearance

Single injection:

The level of citrate in the serum of all animals fell rapidly after the injection and returned to the normal range within 90 – 120 minutes.

Repeated injection:

An initial injection of 1 ml of 15 % sodium citrate, followed by repeated injections of 0.25 ml of the solution, showed no indication of impairment of the normal mechanism for the elimination of the citrate.

Giving repeated doses of 0.5 ml at 30-minute intervals apparently approached the limit of the citrate removing capacity of the rabbit, since each dose raised the level of citrate in the serum to about 16 mg %. The magnitude of the fall in the serum citrate in the 20 minute interval between doses was fairly uniform and amounted to an average of 4.25 ± 0.62 mg %.

4 doses of 1 ml of 15 % sodium citrate at intervals of 30 minutes caused an accumulation of citrate in the blood. This rate of injection, therefore, must have exceeded the capacity to dispose of the citrate and, when the level reached about 25 mg %, the animal suffered convulsions and died within a few minutes.

Summary and Conclusion

The slow intravenous injection of 300 mg of sodium citrate did not cause significant reduction in the ionised calcium of the blood plasma.

The restoration of the calcium ions of the serum after injection of a toxic dose of citrate was very rapid and the concentration was restored nearly to normal within a few minutes.

The level of citrate in the serum of all animals following single injection fell rapidly after the injection and returned to the normal range within 90 – 120 minutes.

Giving repeated doses of 0.5 ml at 30-minute intervals apparently approached the limit of the citrate removing capacity of the rabbit, since each dose raised the level of citrate in the serum to about 16 mg %.

Doses of 1 ml of 15 % sodium citrate at intervals of 30 minutes caused an accumulation of citrate in the blood. This rate of injection, therefore, must have exceeded the capacity to dispose of the citrate and, when the level reached about 25 mg %, the animal suffered convulsions and died within a few minutes.

The maximum tolerance for citrate in rabbits of comparable body weight varies considerably from one animal to another. The rabbit is capable of rapidly clearing and disposing of large amounts of citrate. No effect on calcium ion concentration of the blood could be demonstrated except when a concentrated solution of citrate was injected sufficiently rapidly to produce convulsions. Even under these conditions, the calcium ion concentration in the blood was rapidly restored and usually a complete recovery was made within 5 minutes.

2.1.4 Baruch SB et al. (1975)

Study reference:

Baruch S.B., Burich R.L., Eun C.K., King V.F. (1975), Renal Metabolism of Citrate, Medical Clinics of North America, Vol. 59, No. 3, 569-582

Detailed study summary and results:

The report discusses the renal handling of citrate *in vivo*, i.e. the factors affecting tubular transport, uptake and production of citrate. The experiments have been performed on dogs weighing 20 – 30 kg. The net rates of reabsorption, secretion, uptake, production and peritubular transport of citrate into tubular cells from peritubular blood or in the opposite direction, can be calculated.

Method

A number of groups of animals were studied, as follows:

5 dogs infused with 150 µmole/min of malate
9 dogs infused with 150 µmole/min of citrate
4 dogs with acute metabolic alkalosis (induced by infusion of sodium bicarbonate)
9 dogs with acute metabolic alkalosis infused with 150 µmole/min of citrate
4 dogs with acute respiratory alkalosis (induced by mechanical hyperventilation)
9 dogs with acute respiratory alkalosis infused with 150 µmole/min of citrate
13 dogs with mildmetabolic acidosis, probably caused by high protein content in the diet, acted as controls.

Results

Metabolism and clearance

Renal handling of citrate:

During malate infusion, arterial plasma citrate increased but glomerular filtration rate (GFR) decreased. Malate increased urinary excretion of citrate to exceed the quantity filtered. Thus, there was a net citrate secretion of 1.29 µmol/min, contrasting with net control reabsorption of 3.49 µmol/min. In addition, malate infusion abolished peritubular transport into cells and induced net renal production of citrate.

Effects of alkalosis on renal handling of citrate:

Acute metabolic alkalosis depresses net citrate reabsorption, especially at higher filtered rates. In dogs whose plasma citrate levels were elevated by citrate infusion, alkalosis did not change citrate filtration, but it depressed net reabsorption, increased urinary excretion, caused release of citrate from cells into peritubular blood, abolished overall citrate uptake and raised the renal cortical concentration.

The kidney is the main tissue for citrate clearance from the body and the rate of renal citrate uptake is determined largely by intracellular metabolism rather than by cell membrane transport. During citrate infusion at 150 µmol/min, the control uptake rate for 1 kidney was 40 µmol/min or 80 µmol/min for both kidneys. This is 53 % of the infused rate. Similar calculations for urinary excretion give 15 % of the infused rate. The sum, uptake plus excretion, equals renal extraction which is 68 % of the infused rate.

Citrate content and permeability of red blood cells:

Red blood cells contain citrate but their permeability to citrate is nil and unaltered by chronic acid-base changes. The citrate removed by the kidney from circulating blood therefore derives entirely from the plasma.

Renal utilisation of citrate:

Essentially all the citrate taken up by renal tubular cells is converted to CO_2 and this holds for either acidosis or alkalosis. Approximately 15 % of the total renal production of CO_2 is derived from citrate.

Summary and Conclusions

Studies on renal handling have shown that:

- Citrate enters renal tubules from luminal fluid (reabsorption) and peritubular blood.
- Reabsorption becomes maximal, i.e. Tm-limited, at filtered loads 7-8 times the normal (i.e. above 30 µmole/min).
- Administration of malate stimulates net renal production of citrate leading to release into urine (net secretion) and peritubular blood.
- Acute metabolic alkalosis, induced while plasma citrate levels are above normal, depresses net citrate reabsorption, stimulates citrate release into peritubular blood and abolishes overall renal uptake of citrate.
- Essentially all citrate extracted by the kidney is converted to CO_2 at endogenous circulating levels. This contribution is 15 % of the total renal CO_2 production and is independent of chronic alterations in acid-base balance

2.1.5 REACH registration dossier (2010)

Study reference:

REACH registration dossier (secondary source, 2010)

Detailed study summary and results:

Available data assembled into one report.

Citric acid is a metabolic intermediate vital to the TCA respiration pathway found in all animals and plant cells. There is little evidence that citric acid and the citrate salts have deleterious effects, even in large doses. Indeed there is some support for the fact that citric acid in human diet is favourable by inhibiting the formation of calcium oxalate kidney and bladder stone.

2.1.6 Dzik W.H., Kirkley S.A. (1988)

Study reference:

Dzik W.H., Kirkley S.A. (1988), Citrate toxicity during massive blood transfusion, Transfusion Medicine Reviews, Vol. 2, No. 2, pp 76-94, 1988

Detailed study summary and results:

This report focuses on citrate toxicity during massive blood replacement in adults. The dose, distribution, metabolism and excretion of citrate are discussed.

Exposure regime

Massive transfusion
In one study, 500 ml of citrated blood was infused over 5 minutes to adults (4 mg citrate/kg/min).
In another study, 5 patients received a mean of 5.5 mg citrate/kg/min over 15.6 minutes.

Table 4: The citrate burden of various anticoagulant preservative formulations

Table 2. Citrate Content of Various Anticoagulant–Perservative Formulations

	ACD	CPD/CPDA-1	AS-1	AS-3
Grams trisodium citrate ($2H_2O$)	1.485	1.656	1.656	2.244
Grams citric acid (H_2O)	.540	.206	.206	.248
Grams citrate per unit	1.451	1.261	1.261	1.681
Concentration of citrate per liter (mmol/L)	7.6	6.7	6.7	8.9
Concentration of citrate (mg/dL)* in:				
Whole Blood	280	246	206	274
Packed RBC	87	76	54	181
FFP	436	384	384	384
Quantity of citrate (mg)* in:				
Whole Blood	1451	1261	1261	1681
Packed RBC	200	176	176	596
FFP	976	843	843	843

* Calculated assuming a 450 mL donation; HCT, 41, and no movement of citrate into cells. For ACD, CPD and CPDA-1 assumes the production of packed RBC with Hct, 80; FFP, 230 mL; and platelet concentrate, 55 mL. For AS-1 and AS-3 assumes production of red blood cells with final Hct, 56; FFP, 230 mL; and platelet concentrate, 55 mL.

Results

Dosage and distribution

The level of citrate in the bloodstream during massive transfusion results from the net balance of citrate dose versus citrate removal. Citrate is rapidly removed by the liver and kidney. However, the distribution of citrate also plays an important role in determining the citrate level following transfusion. Although the relative importance of metabolism versus redistribution of citrate is not fully investigated, citrate can be considered as a first order approximation to be distributed throughout the extracellular fluid space. This distribution occurs within 5 minutes of infusions of mild to moderate quantities of citrate. For example, in one study, 500 ml of citrated blood was infused over 5 minutes to adults (4 mg citrate/kg/min x 5). At the end of the infusion the measured citrate level was 66 % of the level that would have been predicted had all the citrate remained in the intravascular space. Within 3 minutes after stopping the infusion, the level of citrate in the blood was equal to that which would have been predicted based on the assumption that citrate would be distributed over the extracellular volume.

However, rapid challenges of large quantities of citrate can exceed/increase redistribution, metabolism and excretion. 5 patients receiving a mean of 5.5 mg citrate/kg/min over 15.6 minutes developed an average peak citrate level of 62 mg/dl which was 1.5 times greater than the expected citrate level, assuming complete extracellular redistribution. As a result of metabolism and redistribution outside the vascular space, there is an initial exponential decline in the concentration of citrate after cessation of rapid blood infusion. Further, removal of citrate results from continued metabolism and renal excretion. Due to the permeability of cells to citrate and the apparent large volume of distribution, complete metabolism and excretion following prolonged rapid transfusion would be expected to take several hours.

Citrate metabolism

The metabolism of citrate involves multiple biochemical pathways. Citrate can directly enter the Kreb's tricarboxylic acid cycle to be completely metabolised to CO_2 and H_2O, participate in fatty acid and amino acid synthesis and be converted to glucose via gluconeogenesis.

Exogenous citrate can be actively transported through the mitochondrial membrane to participate in the Kreb's cycle reactions.

An additional biochemical pathway of citrate metabolism is provided by citrate cleaving enzyme. This enzyme, ATP-citrate lyase, is found in liver and adipose cells and splits cytoplasmic citrate in the presence CoASH to form acetylCoA and oxaloacetate. The acetylCoA formed cannot permeate the mitochondria but is able to participate in the biosynthesis of fatty acids. Thus, increased cytoplasmic citrate as a result of massive transfusion might be expected to temporarily stimulate fatty acid synthesis.

The rate limiting step of citrate metabolism following massive transfusion is unknown.

Citrate clearance

Clearance of citrate is the highest in those organs which receive a high proportion of the cardiac output and which are composed of cells with numerous mitochondria. Cells which are dependent on glycolysis for their energy needs, such as red blood cells, have low levels of citrate and do not remove citrate from the circulation. Liver, kidney and skeletal muscle are responsible for most of the metabolism and excretion of citrate.

In normal fasting humans, about 20 % of the endogenous citrate in the serum is removed with each pass through the liver.

The kidney is also essential for citrate clearance. It is able to metabolise large amounts of citrate and can excrete non-metabolised citrate in the urine. Estimates of the amount of exogenous citrate handled by the kidney range from 20 % to 68 % of a given load in humans. Most of the uptake of citrate by the kidney is from reabsorption of filtered citrate in the proximal tubule but up to 30 % of the total renal uptake is peritubular uptake from postglomerular blood. The proximal tubular cells, which are rich in mitochondria are responsible for the metabolism of citrate. Normally, < 1 % of the citrate filtered by the kidney is excreted in the urine but, in the presence of high plasma concentration of citrate, there is a dramatic increase in the fractional excretion in the urine.

Summary and Conclusion

Dose and distribution:

The level of citrate in the bloodstream during massive transfusion results from the net balance of citrate dose versus citrate removal. Citrate is rapidly removed by the liver and kidney. Although the relative importance of metabolism against redistribution of citrate is not fully investigated, citrate can be considered as a first order approximation to be distributed throughout the extracellular fluid space. This distribution occurs within 5 minutes of infusions of mild to moderate quantities of citrate. Due to the permeability of cells to citrate and the apparent large volume of distribution, complete metabolism and excretion following prolonged rapid transfusion would be expected to take several hours.

Metabolism:

Citrate can directly enter the Kreb's tricarboxylic acid cycle to be completely metabolised to CO_2 and H_2O, participate in fatty acid and amino acid synthesis and be converted to glucose via gluconeogenesis. An additional biochemical pathway of citrate metabolism is provided by citrate cleaving enzyme. This enzyme, ATP-citrate lyase is found in liver and adipose cells and splits cytoplasmic citrate in the presence CoASH to form acetylCoA and oxaloacetate.

Clearance:

Clearance of citrate is the highest in those organs which receive a high proportion of the cardiac output and which are composed of cells with numerous mitochondria. Liver, kidney and skeletal muscle are responsible for most of the metabolism and excretion of citrate.

Conclusion

Due to the permeability of cells to citrate and the apparent large volume of distribution, complete metabolism and excretion following prolonged rapid transfusion would be expected to take several hours.

2.1.7 *Nordmann J., Nordmann R. (1961)*

Study reference:

Nordmann J., Nordmann R. (1961), Organic acids in blood and urine. Adv. Clin. Chem. 4: 53-120

Detailed study summary and results:

The paper is a review of findings regarding citric acid in the blood and urine of humans (adults) up to the date of publication.

Results

Distribution

In 46 normal human subjects, the level of citric acid in blood plasma is approximately 1.54 ± 0.9 mg% citric acid. The level is dependent upon the age of the subject with the level decreasing with age (1.70 ± 0.28 mg% between 11 and 20 years and 1.15 ± 0.24 mg% after 60 years)

The amount of citrate in the blood plasma is correlated with the amount of ionised calcium. An increase in blood citrate reduces ionised calcium.

The paper also discusses the effect on the citric acid cycle of certain diseases.

Metabolism

Metabolism is not discussed as the citric acid cycle is well established

Citrate clearance

Normal urinary excretion of citric acid is 200 to 1000 mg/day. Urinary citrate plays a fundamental role in keeping calcium in solution and preventing precipitation of calcium salts in the urinary tract.

The mechanism of urinary elimination was studied in dogs. Most of the filtered citric acid is reabsorbed in the tubules, the citric acid clearance in the dog being less than 3 % of the glomerular clearance.

Citric acid is present in urine even at birth, before the first feed.

The amount of citrate eliminated in urine is largely dependent upon diet. More citrate is eliminated for example when the diet is rich in carbohydrate. Higher excretion of citric acid occurs in rats after administration of structural carbohydrates rather than those used for energy. The fluctuations in citric acid are mirrored by fluctuations in calcium elimination, so relating calcium elimination to citric acid.

Urinary citrate decreases after administration of substances which produce acidosis, but increases after alkali administration, this mechanism eliminates excess cations without removing fixed anions.

The main determinant of citrate excretion is probably the intracellular pH of the tubular cells.

Hormones and vitamins may also have an effect on urinary excretion of citrates. For example, elimination fluctuates in a regular pattern throughout the menstrual cycle. Vitamin D may also effect the elimination of citrate, administration of the vitamin increasing citrate elimination.

Summary

Citric acid is found in the blood plasma of all humans, the level varying according to the age of the subject.

Citrate is also eliminated in urine of all mammals. The amount being dependent upon several factors including diet, pH of cells, hormone and vitamin levels and also contributory factors such as disease.

Citrate levels are linked to calcium in the body.

Conclusion

Citric acid plays a key role in the body’s natural metabolism processes.

2.1.8 Bunker J.P. et al. (1955)

Study reference:

Bunker J.P., Stetson J.B., Coe R.C., Grillo H.C., Murphy A.J. (1955), Citric acid intoxication, The Journal of the American Medical Association, Vol. 157, No. 16, p 1361-1367, 1955.

Detailed study summary and results:

Sodium citrate, calcium, total protein, magnesium and potassium levels were measured in 130 patients, both surgical and medical, during the transfusion of moderate to large volumes of citrated blood.

Each unit of blood from the Massachusetts General Hospital blood bank contained 75 ml of an acid-citrate-dextrose solution of 1.33 % sodium citrate, 0.47 % citric acid and 3.0 % dextrose. Each unit of blood from the American Red Cross blood bank contained 125 ml of a solution of 1.33 % sodium citrate, 0.47 % citric acid and 1.47 % dextrose.

Results

Dosage and distribution

33 normal adult patients who had major surgery and received multiple transfusions at a rate of not more than about 500 ml every 30 minutes gave no evidence of citric acid intoxication. Thus if the rate at which citrate was infused remained below 0.5 mg/kg bw/min, the serum concentration of citrate ion remained below 9 mg/100 ml and the calculated ionised calcium level remained above 0.85 mM/l, which is within the normal range.

19 of the patients received transfusions of 2000 ml or more of whole blood and 5 received more than 3500 ml. The report data confirms that transfusions of moderately large volumes of citrated blood can be given to normal patients with clinical safety.

At the lower rates of citrate infusion the concentration of serum citrate was roughly proportional to the infusion rate, although there was considerable variation from patient to patient. There was no correlation at these low rates between total infused citrate and the concentration of serum citrate.

In 11 patients, all of whom had normal liver function arterial samples drawn just before and just after the rapid infusion of large amounts of blood (quantity not specified) allowed us to calculate roughly the probable disposition of the infused citrate. 5 patients receiving 0.75 – 1.7 g of citric acid within 6 – 9 minutes retained approximately half in the vascular compartment. 6 patients who received 1 – 3 g of citrate ion in 11 – 16 minutes had apparently already redistributed it through the extracellular space.

Summary

33 normal adult patients who had major surgery and received multiple transfusions at a rate of not more than about 500 ml every 30 minutes gave no evidence of citric acid intoxication.

At the lower rates of citrate infusion the concentration of serum citrate was roughly proportional to the infusion rate, although there was considerable variation from patient to patient. There was no correlation at these low rates between total infused citrate and the concentration of serum citrate.

5 patients receiving 0.75 – 1.7 g of citric acid within 6 – 9 minutes retained approximately half in the vascular compartment. 6 patients who received 1 – 3 g of citrate ion in 11 – 16 minutes had apparently already redistributed it through the extracellular space.

Conclusion

The report discusses the distribution of citric acid and citric acid intoxication. There were marked elevations in serum citrate level sufficient to depress the ionised calcium level seriously. Very high concentrations of serum citrate were observed during multiple transfusions in patients with liver disease or mechanical obstruction to hepatic circulation and in all patients with or without liver disease, during extremely rapid or prolonged infusion of citrated blood or plasma.

2.1.9 Denlinger J.K .et al. (1976)

Study reference:

Denlinger J.K. et al. (1976), Hypocalcaemia during rapid blood transfusion in anaesthetised man, British Journal of Anaesthesia, Volume 48, p 995-1000

Detailed study summary and results:

30 patients were selected for study. Patients were studied without hepatic or renal disease, required radical cancer surgery and presented normal Ca^{2+} levels. Patients were grouped according to the substance administered and the rate of infusion.

Anaesthesia was induced with thiopentone and maintained with enflurane, fluroxene or halothane in 50 % nitrous oxide in oxygen. Body temperature, arterial and central venous pressures and ECG were monitored continuously.

Control measurements of Ca^{2+}, pH and Pa_{CO2} were measured in 5 groups of patients prior to transfusion.

Citrated whole blood was administered for 5 minutes at controlled rates of 50, 100 and 150 ml/70 kg/min.

Table 5: Control measurements of Ca2+, pH and PaCO2

TABLE I. *Control measurements of Ca^{2+}, pH and P_{aCO_2} in five groups of patients*

Group	No. patients	I.v. infusion	Infusion rate (ml/70 kg/min)	Control measurements* Ca^{2+} (m mol/litre)	pH (units)	P_{aCO_2} (mm Hg)
I	5	Citrated whole blood	50	1.07±0.03	7.38±0.02	39±2
II	10	Citrated whole blood	100	1.07±0.04	7.43±0.01	37±1
III	5	Citrated whole blood	150	1.08±0.04	7.42±0.02	43±2
IV	5	Normal saline	100	1.08±0.02	7.39±0.02	42±4
V	5	5% plasma protein fraction	100	1.04±0.01	7.44±0.04	41±3
Total	30			1.07±0.01	7.41±0.01	40.8±1.0

* Mean±SEM.

Samples were obtained at 1 minute intervals during the 5 minute infusion period, for 5 minutes thereafter and at 10 minutes after completion of the infusion

Arterial samples for calcium and citrate analysis were obtained.

Results

Dosage and distribution

Ca^{2+} decreased significantly from the control value during blood transfusion at each flow rate studied. The maximum decreases from control were 14, 31 and 41 % at infusion rates of 50, 100 and 150 ml/70 kg/min respectively. Ca^{2+} returned rapidly to near normal values after blood transfusion. The mechanism of acute calcium rebound may be explained on the basis of rapid mobilisation of calcium ions from skeletal stores, renal and hepatic clearance of citrate or simple redistribution of exogenous citrate in extracellular fluid.

In this case it is doubtful that the skeletal mobilisation of calcium is responsible for the acute calcium rebound. However, there was a similarity between the time course of Ca^{2+} and citrate changes, suggesting that increase in Ca^{2+} is a result of redistribution of citrate in extracellular fluid or citrate clearance by the liver and kidney, or both.

Clearance

The report states that citric acid is cleared by the liver and the kidney.

The isolated liver is capable of removing nearly 100 times the normal concentration of plasma citrate from a perfusate with a large citrate concentration in a single passage. Renal citrate excretion has been measured in dogs with increased citrate concentrations in the blood.

Summary

Distribution:

Ca^{2+} decreased significantly from the control value during blood transfusion at each flow rate studied. Ca^{2+} returned rapidly to near normal values after blood transfusion. The mechanism of acute calcium rebound may be explained on the basis of rapid mobilisation of calcium ions from skeletal stores, renal and hepatic clearance of citrate or simple redistribution of exogenous citrate in extracellular fluid.

Clearance:

The isolated liver is capable of removing nearly 100 times the normal concentration of plasma citrate from a perfusate with a large citrate concentration in a single passage. Renal citrate excretion has been measured in dogs with increased citrate concentrations in the blood.

Conclusion

Administration of citrated whole blood resulted in decreases in the calcium ion concentration, which then returned rapidly to control values after termination of the transfusion. Reciprocal changes in serum citrate concentrations occurred, suggesting that the transient hypocalcaemia was a result of redistribution of citrate and hepatic or renal clearance from the vascular space. Hypocalcaemia accompanying blood transfusion is a transient phenomenon dependent on the total dose of citrate administered and the rate of infusion.

2.1.10 Howland W.S. et al. (1957)

Study reference:

Howland, W.S. et al. (1957), Massive blood replacement. Surg. Gynaecol. Obslet. 105: 529-540

Detailed study summary and results:

This report focuses on citrate toxicity during blood replacement in adults before, during and after surgery and in parallel of an anticoagulant solution infusion containing citric acid. The dose, distribution, metabolism and excretion of citrate are discussed.

Method

Acid citrate dextrose acid solution is used as an anticoagulant for stored blood. The solution contains 13.2 g trisodium citrate, 4.9 g citric acid and 14.7 g dextrose diluted to 1000 ml with distilled water. Blood administered in the study contained 120 ml of solution per bottle, equivalent to 1.43 g citric acid per unit of blood (1 unit of blood equals 480 ml blood plus 120 ml anticoagulant solution).

In iv administration of only the anticoagulant, the solution was administered at rates of 40 ml/min (8.6 mg/kg/min citric acid) for 20 to 30 minutes.

The following patients (adults) were considered:

Normal levels (control): 120 patients
Patients with abnormal liver function: 2 patients
Anticoagulant iv only: 3 patients, clotting studies on 1 patient only
Blood transfusion: 77 patients, urine analysis on only 3 patients receiving high levels of blood.
Pregnant women: 6 in 1st trimester, 54 in 2nd and 55 in 3rd.

Plasma citric acid levels were determined in preoperative patients during and after blood transfusion and during gestation.

Normal values were obtained from 120 patients

Results

Dosage and distribution

Mean normal plasma citric acid levels were found to be 0.8 ± 0.8 mg%for men and 1.08 ± 0.9 mg%for women.

In the presence of liver disfunction, mean citric acid levels are not significantly elevated. The mean value for these patients was 0.57 ± 0.47 mg%.

There was a variation in plasma levels during the 3 stages of pregnancy:

1st trimester: 2.36 ± 2.5 mg%.

2nd trimester: 2.04 ± 1.5 mg%.

3rd trimester: 1.4 ± 0.27 mg%.

The 3 patients receiving iv anticoagulant solution received an equivalent of citric acid at rates of 6.25 to 8.6 mg/kg/min. all patients showed a marked increase in citric acid levels. The peak coincided with termination of the administration. In the studied patient, the elevation was followed by a progressive fall until an almost normal level was obtained 40 minutes after the injection (60 minutes after start of the experiment). There were no significant changes in the clotting determinations carried out on this patient.

Patients receiving citrated blood during transfusions showed considerable variation. Where blood was administered slowly, plasma citric acid levels were not often significantly increased. Patients receiving blood rapidly invariably had high plasma citric acid levels. The rate of transfusion was the most important factor in determining peak levels of plasma citric acid rather than the amount given.

For a rate of infusion of citric acid of up to 8.6 mg/kg/min, plasma citric acid was elevated 8 mg/kg% for every mg/kg/min citric acid infusion.

Citrate metabolism

Citric acid occurs endogenously and is part of Kreb's cycle. Its precursors are oxalacetate and "active" acetate. A large quantity of citric acid is metabolised by muscle, the main site of carbohydrate metabolism, and it is readily metabolised through the stages of cisaconitic and isocitric acid in the presence of the enzyme aconitase. A daily intake of 40 g citric acid is oxidised completely in the body and is normally excreted in the urine at a rate of 0.20 to 1.0 g /day

When large amounts of citric acid are infused, it is possible that the normal enzyme systems metabolising citric acid becomes saturated. The only patient in which potential saturation may have occurred had a peak value of 173 mg%but this was in samples from the vena cava and may not represent a mean plasma citric acid value.

The liver is observed to not be the most important site of citric acid metabolism.

The ability to recover from the toxic effects of citric acid is chiefly due to rapid metabolism not to excretion in urine. The kidney is not the main pathway for elimination of citric acid.

Citric acid produces toxic effects by lowering the concentration of plasma ionised calcium. Citrate intoxication is more likely to occur in those with impaired calcium metabolic processes.

Citrate clearance

In patients treated by transfusion of citrated blood, the turnover time of citric acid was very short.

Urine specimens obtained for 3 days after infusion did not show a marked increase in urine citric acid excretion.

Summary

Dose and distribution:

Mean normal plasma citric acid levels were found to be 0.8 ± 0.8 mg%for men and 1.08 ± 0.9 mg% for women.

In the presence of liver disfunction, mean citric acid levels are not significantly elevated. The mean value for these patients was 0.57 ± 0.47 mg%.

Increases in plasma citric acid concentrations are directly related to the rate of infusion not to the amount infused.

Metabolism:

Metabolism is very rapid, plasma citrate levels are normal within 90 minutes of administration. The liver does not have an effect on metabolism. Citrate intoxication is linked to dysfunctional calcium metabolic processes.

Clearance:

The kidney is not a main pathway of elimination. High plasma concentrations do not induce a significant increase in elimination in urine.

Conclusion

Citric acid metabolism is rapid (approx. 90 minutes). Metabolic processes are not affected by the liver or kidney. Increases in citric acid levels are determined by the rate of infusion not by the amount of citric acid infused.

2.1.11 Ludbrook J., Wynn V. (1958)

Study reference:

Ludbrook J., Wynn V. (1958), Citrate Intoxication: a clinical and experimental study, British Medical Journal, p 523-528

Detailed study summary and results:

Treatment

Case 1:

A man of 55 was operated under induced hypothermia for an aortic thrombosis extending up to the level of the renal arteries. The patient received early in the operation 2.2 L of blood infused over 1 h (1.4 mg citrate/kg/min) exactly replacing blood loss. After release of the aorta clamps, 3.25 L of blood was infused over 1.4 h (1.5 mg citrate/kg/min).The patient died in ventricular fibrillation after receving a total of 7.5 L of blood (26 g citrate).

Case 2:

A man of 35 was operated for a syphilitic aortic aneurysm. The aorta was clamped during the operation, above and below the aneurysm. In the approx. 1 hour period before clamping, the ctrate infusion rate was 0.4 mg/kg/min, increasing to 0.6 mg/kg/min during the clamping period. At the end of the period of aortic clamping the plasma citrate level had risen to 41 mg/100 ml and immediately after release of the clamps to 48 mg/100 ml. During an equal period following release of the clamps with an identical rate of citrate infusion the plasma citrate fell to 13 mg/100 ml.

Case 3:

The patient was a woman of 44 with a thrombosis of the abdominal aorta extending up to just below the renal arteries. During 30 minutes, after the clamps were released, 1100 ml of blood was infused (1.9 mg citrate/kg/min). The plasma citrate rose to 39.5 mg/100 ml and the calculated rate of citrate removal from the plasma was 65 % of normal.

Case 4:

A man of 24 underwent partial hepatectomy for a haemangloma of the liver with associated cirrhosis. The patient received the following doses:

preoperative = 0
15 min after clamping hepatic artery = 0.4 mg citrate/kg/min
15 min after unclamping = 1.2 mg citrate/kg/min
1 h post-operative = 0.85 mg citrate/kg/min

Results

Dosage and distribution

In normal human blood, the citrate ion is virtually confined to the plasma. It is possible to show that at least 98 % of the added citrate remained in the plasma. In such blood, there was over a period of 3 weeks a progressive decrease in plasma citrate concentration to 92 % of the original level but whether this represents metabolic destruction of citrate or a movement into the red cells was not determined.

Metabolism

In man, metabolic destruction is more important as a mean of removing citrate from the plasma than in the dog. For a given elevation of plasma citrate, the rate of metabolic destruction in man is about twice that in the dog weight for weight.

Hypothermia in man to 28 – 29 °C reduced the rate of metabolic destruction of citrate by 30 – 40 %.

Clearance

In normal unanaesthetised man, only 18 to 20 % infused citrate was excreted in the urine. The remainder was presumably metabolically destroyed. The urine-plasma concentration ratio ranged from 14 – 25 in the human.

High plasma citrate levels are unlikely to occur with citrated blood transfusions in normal humans, except where the rate of infusion is extremely rapid (greater than 540 ml per 5 minutes in an adult or during rapid exchange transfusion in infancts). In metabolically abnormal patients with liver disease for example, the ability to clear the plasma of citrate may be so impaired as to make high plasma citrate levels a real danger

Summary

In normal human blood, the citrate ion is virtually confined to the plasma. It is possible to show that at least 98 % of the added citrate remained in the plasma.

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High plasma citrate levels are unlikely to occur with citrated blood transfusions in normal humans, except where the rate of infusion is extremely rapid (greater than 540 ml per 5 minutes in an adult or during rapid exchange transfusion in infancts).. In metabolically abnormal patients with liver disease for example, the ability to clear the plasma of citrate may be so impaired as to make high plasma citrate levels a real danger.

Conclusion

High plasma citrate levels occurred during the infusion of citrated blood into 4 patients undergoing surgery under hypothermia. One patient died with signs attributable to citrate intoxication.

A linear relationship between the rate of infusion of citrate and the rise in plasma citrate level was demonstrated in man. For each 1 mg of citrate infused/kg/min the rise was about 12.5 mg/100 ml in humans.

By using a constant infusion technique it was shown in man that 20 % of infused citrate was excreted in the urine and the remainder was metabolically destroyed.

Hypothermia in man to 28 – 29 °C reduced the rate of metabolic destruction of citrate by 30 – 40 %.

2.1.12 Abbott T.R. (1983)

Study reference:

Abbott T.R. (1983), Changes in serum calcium fractions and citrate concentrations during massive blood transfusions and cardiopulmonary bypass, British Journal of Anaesthesia, Vol. 55, p 753-759.

Detailed study summary and results:

Treatment

5 children undergoing open heart surgery and 3 children undergoing scoliosis were used in the study.
Scoliosis:
Age: 12 – 14 years
Weight: 39 – 42 kg
Open heart surgery:
Age: 6 – 9 years
Weight: 16 – 25 kg

In all patients the blood volume was maintained by transfusing CPD blood.

Those undergoing scoliosis surgeries had 95 %, 79 % and 100 % of their blood replaced.

In the group undergoing open heart surgery the oxygenator prime consisted of 0.18 % saline in 5 % dextrose plus CPD blood.

Blood samples were drawn at 15 min intervals in the patients undergoing spinal surgery.

In the group undergoing open heart surgery, samples were taken from the patient before, during and immediately after cardiopulmonary bypass and then at hourly intervals for 5 h.

Results

Dosage and distribution

Open heart surgery:

During bypass, total calcium, citrate and complexed calcium increased markedly as a result of the mixing of the blood from the oxygenator prime with the patient's blood. During the period after operation, citrate and complexed calcium concentrations continued to decrease but were still greater than 5 times normal after 6 h. Although post- induction and pre-bypass values were normal for Patient 5, citrate concentrations failed to decrease as rapidly as in the other patients and ionised calcium remained low for 24 h. This patient eventually died.

Scoliosis surgery:

Patient 7 received two increments of 16 % of blood volume over 5 min and patient 8 two increments of 10 % of blood volume each over 5 min periods. In these 2 cases there were acute increases in the concentrations of complexed calcium and citrate and a coincidental decrease in the ionised and protein bound calcium concentrations. Recovery of these values took 2-3 h.

Metabolism

Metabolic destruction is the most important mean to remove citrate from the plasma and for its plasma concentrations to return to normal over a period of about 1 h following the transfusion. In the present study, citrate concentrations took about 2 h to return to normal in the spinal surgery patients.

Clearance

In patients undergoing open heart surgery, clearance of citrate from the blood was decreased since all were moderately hypothermic and since they had all undergone complex intracardiac surgery, cardiac output may have been decreased also. The rate of clearance of serum citrate was decreased in one patient and was associated with a persistently low calcium ion concentration for more than 24 h following surgery.

Clearance of citrate from the blood will be decreased during hypothermia if liver function is impaired and will be increased if hypothermia is corrected, systemic and hepatic blood flow increased and urine output increased. Approximately 20 % of citrate can be excreted in the urine.

Summary

Total serum calcium, its fractions and serum citrate concentrations were measured in children receiving massive blood transfusion for scoliosis surgery and in other children undergoing open heart surgery with cardiopulomonary bypass.

During the period after open heart surgery citrate and complexed calcium concentrations continued to decrease but were still greater than 5 times normal after 5 h.

In patients undergoing open heart surgery, clearance of citrate from the blood was decreased since all were moderately hypothermic and since they had all undergone complex intracardiac surgery, cardiac output may have also been decreased.

In the present study citrate concentrations took about 2 h to return to normal in the spinal surgery patients.

Clearance of citrate from the blood will be decreased during hypothermia if liver function is impaired and will be increased if hypothermia is corrected, systemic and hepatic blood flow increased and urine output increased. Approximately 20 % of citrate can be excreted in the urine.

Conclusion

During scoliosis surgery, serum ionised calcium concentration remained within normal limits, provided that infusion rates of blood did not exceed 30 ml/kg/h. During cardiopulmonary bypass, serum ionised calcium concentrations decreased markedly during perfusion associated with extremely high serum citrate concentrations. Serum ionised calcium concentrations returned to normal by the end of the bypass but serum citrate concentrations remained 5 times the normal concentration 5 h after bypass.

2.1.13 Driscoll D.F. et al. (1987)

Study reference:

Driscoll D.F. et al. (1987), Development of Metabolic Alkalosis after Massive Transfusion during Orthotopic Liver Transplantation, Critical Care Medicine, Vol. 15, No. 10, p 905-908.

Detailed study summary and results:

Five patients undergoing orthotopic liver transplantation were investigated for changes in acid-base homeostasis secondary to large volume transfusions.

Treatment

Table 6: Blood product usage in five hepatic transplant recipients

Patient No.	Packed RBC (0)[a]	Fresh Frozen Plasma (14.0)[a]	Platelets (3.0)[a]	Cryoprecipitate (1.0)[a]	Total Citrate Administered (mEq)
1	47	53	67	20	963
2	30	34	50	0	626
3	41	56	50	2	936
4	22	37	30	20	628
5	19	34	60	20	676
Mean ± SD	31.8 ± 12.1	42.8 ± 10.8	51.4 ± 13.9	12.4 ± 10.4	766 ± 195

[a] Estimated milliequivalents of citrate per U of blood product based on 17 mEq of citrate/donor bag.

Each donor bag contains approximately 17 mEq of citrate in the anticoagulant preservative solution.

Each of the 5 patients studied received over 600 mEq of citrate with an estimated mean amount of 750 mEq.

The individual clinical course of each patient was analysed for etiologies contributing to the development of metabolic alkalosis. They included: concurrent drug therapy that could lead to metabolic alkalosis; ventilatory status that could influence acid-base homeostasis; and number and type of blood product infusions. Additional data included arterial blood gas profiles which documented the metabolic changes immediately before, during and after hepatic transplantation.

Results

Each patient exhibited transient acidemia, followed by protracted alkalemia. It took a mean of 10.3 h from the beginning of surgery to achieve the peak arterial pH. The mean peak pH was 7.51, with a high value of 7.56.

Metabolism

Not all of the administered citrate was metabolised by the recipient since some of the citrate-rich bank blood was removed by suction at the operative site. Nevertheless, there was a significant elevation in blood citrate levels.

Table 7: Citrate levels in a group of liver transplant patients

Patient No.	Citrate (mmol/L)		
	First H	Peak	Last H
6	0.3	6.9	3.7
7	0.1	1.3	0.8
8	1.0	1.4	0.3
9	0.3	1.3	1.3
10	1.1	1.1	0.6
11	1.8	4.3	1.5

The citrate levels during the last hour (H) suggest rapid metabolism of the citrate initially after perfusion; however, the persistence of systemic alkalemia after peak levels are reached suggests that the rate slows. The mean value during the last hour of liver transplantation would represent a substantial amount of citrate still to be transformed metabolically if the volume of distribution of citrate is in total body water.

Sodium citrate is almost completely dissociated at physiological pH. For each mole of citrate metabolised, 3 mole of bicarbonate are generated. If this process were to continue unimpeded, the arterial pH would continue to increase and the patient would succumb to the potentially lethal alkalemia. However, this does not occur and the rate limiting step involves the hepatic metabolism of citrate.

The oxidation of citrate via the Krebs cycle as well as its conversion to glucose are the proton consuming processes that lead to alkalosis. It is important to note that the citrate concentrations used in this study are not rate-limiting and with the postabsorptive glucose production rates of 200 g/day, the gluconeogenic capacity to clear citrate is not likely to be rate-limiting since 750 mEq represents approximately 140 g of citrate. Rather, the pH of arterial blood appears to be a key factor regulating citrate metabolism and the development of severe metabolic alkalosis; when systemic pH exceeded 7.5 as in these patients, citrate and lactate metabolism appeared to slow

Clearance

The quality of the liver allograft is important in the development of metabolic alkalosis coincident with reperfusion because its capacity to clear citrate from plasma is highly dependent on good hepatocellular function. A poor liver allograft may delay the development of alkalosis

Summary

All patients developed a transient acidemia during the operative period, followed by alkalemia which persisted into the early postoperative period. The patients received an estimated mean of 750 mEq of citrate, which appeared to cause metabolic alkalosis.

The pH of arterial blood appears to be a key factor in this study regulating citrate metabolism and the development of severe metabolic alkalosis; when systemic pH exceeded 7.5 as in these patients, citrate and lactate metabolism appeared to slow.

Conclusion

The biochemical basis underlying the regulation of citrate metabolism that may have led to the timing, extent and duration of the subsequent metabolic alkalosis is presented. The time course for the development of metabolic alkalosis may be a potentially sensitive indicator of early allograft function.

2.1.14 Simpson D.P. (1983)

Study reference:

Simpson D.P. (1983), Citrate Excretion: A Window on Renal Metabolism, Am. J. Physiol., 244 (Renal Electrolyte Physiol. 13): F223-F234.

Detailed study summary and results:

The paper discusses the excretion and metabolism of citrate and the factors which affect them. Humans, rats and dogs are discussed.

Results

Dosage and distribution

Citrate levels in the plasma of humans and other mammals range between 0.05 and 0.3 mM. Citrate is extensively reabsorbed in the nephron, predominantly in the proximal segments of the tubule. Studies in humans, dog, rat and other species have shown that more citrate disappears into the kidney than can be accounted for by the metabolism of all the citrate reabsorbed in the tubules.

The existence of a citrate carrier on the tubular side of cells of the renal cortex has recently been confirmed.

To reach the enzymes of the citric acid cycle, which are located in the mitochondrial matrix space, citrate must pass across the inner mitochondrial membrane. Citrate is transported by the tricarboxylate carrier and malate can serve as a counter ion

Metabolism

The citrate that disappears into the kidney is metabolised chiefly by the abundant mitochondria of the cells of the proximal convoluted tubules. CO_2 and glucose are the major end-products of citrate metabolism in the kidney. One-third to one-fifth of the citrate supplied to the kidney in the arterial blood is metabolised. Typical renal handling of citrate in humans is as follows: 44 µmol of citrate enter the kidney each minute in the arterial plasma. Each minute 8.8 µmol are filtered of which 75 % or 6.6 µmol are reabsorbed and 25 % or 2.2 µmol are excreted. About 1.5 µmol of citrate are removed from the peritubular blood so that a total of 8.1 µmol of citrate are taken up and metabolised by the kidney each minute.

The effect of metabolic alkalosis on renal citrate handling (increased citrate excretion) arises from inhibition of citrate metabolism in cells of the renal cortex rather than from stimulation of intracellular synthesis of citrate.

Clearance

In humans 10 – 35 % of filtered citrate is excreted in the urine, a much larger fraction than in rats (3 – 7 %) and dogs (< 5 %).

Metabolic alkalosis increases citrate excretion. In humans, as much as 60 % of filtered citrate may appear in the urine during alkalosis. In dogs and rats, citrate excretion may increase by as much as 20 fold.

Metabolic acidosis causes an acute decrease in citrate excretion. In humans, citrate excretion fell by 20 – 50 %. In rats, citrate excretion fell by over 90 %.

These changes in citrate clearance induced by alkalosis or acidosis are accompanied by concomitant changes in citrate concentration in the renal cortex.

Organic acids like malate, succinate and fumarate increase citrate excretion.

Administration of inhibitors of the citric acid cycle may also result in increased citrate excretion, presumably by blocking intrarenal citrate metabolism, increasing intracellular citrate levels and blocking citrate reabsorption.

Summary

Table 8: Conditions affecting citrate excretion

Group	↑ Citrate Excretion	↓ Citrate Excretion
Acid-base changes (and related influences)	alkalosis	acidosis K^+ depletion acetazolamide volume expansion (3, 5) starvation (43, 79)
Organic acids	malate succinate fumarate	
Metabolic inhibitors	fluorocitrate fluoroacetate (dog) malonate maleate	
Miscellaneous	calcitonin (39) lithium (6, 7, 13) vitamin D (11, 12) calcium (human) (28, 56, 104, 110) [or no effect (19)] magnesium (98)	heat (42) ethacrynic acid (16) menstrual period (human) (105) calcium (dog) (21)

During normal conditions of acid-base balance there is a steady removal of citrate from both the tubular and peritubular surfaces of cells of the nephron, predominantly in the proximal convoluted section. Citrate

entering the cell by means of carriers accumulates in the cytoplasm to levels considerably in excess of those in the plasma. Cytoplasmic citrate is transported into mitochondria by the tricarboxylate carrier. After entry into mitochondria, citrate is disposed of by pathways of oxidative metabolism. The rate of citrate metabolism by these pathways is sufficient to cause net renal citrate utilisation that exceeds the rate of citrate reabsorption and consumes citrate from the peritubular side of the cell as well. Some filtered citrate escapes reabsorption and appears in the urine to an extent that varies with different mammalian species. The rate of citrate transport and excretion are affected by the factors given in the table above.

Conclusion

The rate of intracellular metabolism of citrate plays a major role in determining the amount of citrate excreted in the urine. Factors which affect the rate of citrate transport and excretion have been discussed.

3 HEALTH HAZARDS

Acute toxicity

3.1 Acute toxicity - oral route

3.1.1 Animal data

3.1.1.1 Yokotani H. et al. (1971)

Study reference:

Yokotani H. et al. (1971), Acute and Subacute Toxicology Studies of TAKEDA-Citric Acid in Mice and Rats. Journal Takeda Research Laboratory, 30 (1): page 25-31.

Detailed study summary and results:

This study is a published report which details work on acute and subacute toxicology. The report does not specify if any guidelines were followed, however the method is similar to OECD 401.

The report compares TAKEDA-citric acid or NCP for short (which is a refined product of citric acid, produced by fermentative procedure of yeast using normal paraffins as nutrient carbon source) with commercially available citric acid.

In the acute toxicity test, 4-week old male ICR-JCL mice, weighing 20 to 24 g and 5-week old male SD-JCL rats, weighing 110 to 140g were used. The animals were kept in air-conditioned room allowing free access to a commercial diet and drinking water. The solutions of test compounds in desired concentrations were administered orally in volumes of 0.5 ml/10 g bw and 2 ml/100 g bw to mice and rats, respectively. There were 6 animals per dose group.

Behaviour and mortality were observed for 7 days after administration and the LD_{50} values were calculated by the method of Litchfield and Wilcoxon.

Species	Mice and rats
Strain	ICR-JCL(mice) SD-JCL (rats)
Source	No data
Sex	Male
Age/weight at study initiation	4-week old mice weighing 20-24g and 5-week old rats weighing 110-140g.

Number of animals per group	6
Control animals	Yes
Administration/ Exposure	Oral
Postexposure period	7 days
Concentration	Mice: 4820, 5790, 7000 mg/kg bw
	Rats: 10420, 12500, 18000 mg/kg bw
Vehicle	No data
Concentration in vehicle	n/a
Total volume applied	2 ml/kg bw
Controls	Commercially available citric acid used as a 'control'
Examinations	Behaviour and mortality were observed for 7 days after administration

Reliability : 2

Results

One animal given 4820 mg/kg bw (mice) or 10420 mg/kg bw (rats) of NCP died at 20 hours. Deaths from respiratory failure were reported between 20 to 180 minutes in mice dosed at 5790 or 7000 mg/kg bw and rats dosed at 12500 or 18000 mg/kg bw. No further information on the mortality rate per dose is provided in the report. The animals which survived the respiratory failure began to recover gradually within several hours and showed no toxic signs 24 hours later.

Pathology: No abnormalities were found at autopsy except the presence of the haemorrhage of the gastric mucosa.

The report describes the effects of the Takeda-citric acid but only gives the LD_{50} for the commercially available citric acid. Both substances are likely to be similar to that used by the applicant so both will be reported.

NPC LD_{50} in the mouse = 5790 mg/kg bw (4990-6720 mg/kg bw 95% confidence limits)

NPC LD_{50} in the rat = 11700 mg/kg bw (10080-13570 mg/kg bw 95% confidence limits)

Commercially available citric acid LD_{50} in the mouse = 5040 mg/kg bw (4520-5665 mg/kg bw 95% confidence limits)

Conclusion

This study forms part of a body of evidence for acute oral toxicity. From this report it is inferred that LD_{50} for citric acid will be around 5790 mg/kg bw in the mouse and 11700 mg/kg bw in the rat.

3.1.1.2 [redacted]. (1975)

Study reference:

[redacted] *(1975), Summary of Mutagenicity Screening Studies: Host Mediated Assay Cytogenetics, Dominant Lethal Assay. Contract FDA 71-268, Compound FDA 71-54 Citric Acid. Litton Bionetics, Inc*

Detailed study summary and results:

Groups of five Sprague-Dawley rats were dosed by gavage with citric acid in 0.85 % saline at 100, 250, 500, 1000, 2000, 3000 or 5000 mg/kg bw. The test animals were observed for ten days after administration of the citric acid. LD_{50} was calculated using the Litchfield-Wilcoxson method

Test material	Citric acid
Lot/Batch number	USP Control Number 121M231 IV
Test Animals	
Species	Rats and mice
Strain	Sprague-Dawley (rat); ICR (mice)
Source	Flow Laboratories
Sex	male
Age/weight at study initiation	250-419 g (average; rat); 30 g (average; mice), 10-12 weeks old
Number of animals per group	Test I: 10 rats Test II: 10 rats Test II: 5 rats Test II: 6 mice
Control animals	No data
Exposure period	Test I: Single dose (rat) Test II: Single dose (rat) Test II: Daily for 5 days (rat) Test II: Daily for 5 days (mouse)
Postexposure period	Test I: Not applicable (all animals died on Day 1 or Day 2) Test II: 7 days (rat) Test II: 15 days (rat) Test II: 14 days (mouse)
Type	Gavage
Concentration	Test I: 100; 250; 500; 1000; 2000; 3000 and 5000 mg/kg bw (rat) Test II: 5000 mg/kg bw (rat) administered as 43.7 % suspension Test II: 3585, 4019, 4050, 5000 and 5600 (rat) Test II: 3000, 3400, 3900, 4400 and 5000 (mouse)
Vehicle	0.85 % saline

Reliability : 2

Results

Test I: Ten male animals (250 g average bodyweight) received 5000 mg/kg bw citric acid suspended in 0.85 % saline by intubation. Four animals died on day one and the remaining six on day two and necropsy findings were that white fluid was found in the intestine and fluid in the pleural cavity. Dose levels of 100, 250, 500, 1000, 2000 and 3000 mg/kg were selected to determine an acute LD50 of 1700 mg/kg using the Litchfield-Wilcoxson method.

Mortality :

Dose (mg/kg)	# dead / # animals	Day of death and necropsy
100	0/5	None
250	0/5	None

500	1/5	Day 3 (1) : white fluid in the intestine and some pleural fluid
1000	2/5	Day 2 (2) : white fluid in the intestine and some pleural fluid
2000	3/5	Day 1 (1) and day 2 (2) : white fluid in the intestine and some pleural fluid
3000	3/5	Day 2 (3) : white fluid in the intestine and some pleural fluid
5000	10/10	Day 1 (4) and day 2 (6) : white fluid in the intestine and some pleural fluid

Test II (single dose): Citric acid was prepared as a 43.7 % (w/v) suspension in 0.85 % saline and administered orally (method not stated) to 10 male rats (average 350 g bodyweight) at a single dose of 5000 mg/kg. Observation period 7 days. No signs of toxicity or abnormal behaviour were observed in the seven-day observation period. No deaths occurred. At termination all animals were killed and on necropsy no gross findings were observed. The acute oral LD50 is considered to be > 5000 mg/kg bw/day.

Test II (rat): Citric acid was administered orally (method not stated) as a solution in 0.85 % saline to five male rats (419 g average bodyweight) at 3585, 4019, 4050, 5000 and 5600 mg/kg daily for five days. Signs of toxicity and abnormal behaviour included mild loss of activity and hair coat mildly untrifty with signs becoming more severe as the observation period progressed. Total period of observation 15 days when animals were terminated and gross necropsies performed. No abnormal gross findings observed. LD50 (15 day subacute) 4696 mg/kg.

Test II (mice): Citric acid was administered by stomach tube as a solution in 0.85 % saline to six male mice (30 g average) at 3000, 3400, 3900, 4400 and 5000 mg/kg bw once per day for five days. Signs of toxicity and abnormal behaviour included mild loss of activity, untriftiness (sic) and laboured respiration usually starting around day 3. Total period of observation was 14 days when the animals were terminated and gross necropsies performed. No abnormal gross findings were observed. LD50 (14 day subacute) 2409 mg/kg.

Remark :

The LD50 value, in rats, 1700 mg/kg in TEST I is noticeably different compared to the LD50 value of 5000 mg/kg in TEST II. In that latter experiment it was stated that "No signs of toxicity or abnormal behaviour were observed in the seven-day observation period" and that no deaths occurred. However, in the first experiment nine rats had died within three days of dosing, one even at 500 mg/kg. The studies overall (reported in 1975) are not described in great detail and it is now very difficult to ascertain the precision with which they were performed (they were pre-guideline or GLP). However, given the obvious difference between the result from the first experiment to the second experiment, the other rat LD50 values available and mouses LD50 values, it seems highly likely that a technical variable or artefact was most likely the reason for this one demonstrably low value. To emphasise this point, in the same study report (Wier, 1975), a 15-day subacute study (male rats dosed for five days) gave an LD50 value of 4696 mg/kg (4181 to 5541 mg/kg confidence limits).

3.1.1.3 OECD (2001)/ REACH Registration dossier (2010)

Study reference:

OECD (2001), Citric acid: CAS No. 77-92-9 OECD ICCA HPV programme (SIDS dossier) UNEP Publications.

REACH registration dossier (study report, 1981)

Detailed study summary and results:

These data are from an OECD document which was itself a summary of existing data on the acute oral toxicity of citric acid, thus does not contain details of the methods used in the study. This study was not GLP and no method guidelines are mentioned in the summary report, however it is similar to OECD 401.

Test material	Citric acid
Purity	>99%
Test Animals	
Species	Mouse
Strain	SPF albino
Source	No data
Sex	Male and female
Age/weight at study initiation	No data
Number of animals per group	5/sex/group. 60 animals in total
Control animals	Yes
Postexposure period	10 days
Type	Gavage
Concentration	3000, 4343, 6000, 8485, 12000 mg/kg bw.
Vehicle	Food grade tap water
Concentration in vehicle	See above
Total volume applied	20 ml/kg corresponding to 0.4 ml per animal
Controls	0.4 ml tap water administered by gavage.
Examinations	Clinical symptoms were observed 2 hours and 4 hours after administration.
Method of determination of LD_{50}	LD_{50} was calculated using probit method analysis and rounded to the nearest 100 mg value.

Equivalent or similar to OECD 401

Reliability : 2 (assessment in the SIDS dossier)

Results

LD_{50} = 5400 mg/kg bw, 95 confidence interval = 4500-6400 mg/kg.

All mortalities occurred in the first 24 hours after administration.

Table 9: Summary of effects*

Dose (g/kg bw)	Mortality (male + female)	Time of death (within)	Toxicity	
			At 2 hours	At 24 hours
0	0/10	/	None	None
3	0/10	/	None	None
4.2	2/10	24 h	None	None
6	7/10	24 h	Slight relaxation	None

8.5	10/10	24 h	Death	/
12	10/10	24 h	Death	/

* Taken from REACH registration dossier (study report, 1981)

Conclusion

The LD_{50} to mice of 5400 mg/kg bw suggests that citric acid is of low toxicity to mice. These data are to be used in conjunction with other existing literature data on citric acid which also indicates the LD_{50} in mice is >5000 mg/kg bw.

3.1.2 Human data

3.1.2.1 Demars CS (2001)

Study reference:

Demars C.S. et al. (2001), Citric Acid Ingestion: A life-threatening cause of metabolic acidosis, Annals of Emergency Medicine, Vol. 28 (5), p 588-591, ISSN 0196-0644

Detailed study summary and results:

A 42 year-old previously healthy male prisoner was brought to the ED by guards because he drank a large volume of a commercial solution of unknown composition sometime in the preceding 6 hours. Apparently he did not have access to other toxins. His medical history was non-contributory except for the presence of severe epigastric pain after this ingestion.

Diagnosis was established once the composition of the ingested fluid, DepotPac toilet bowl and bathroom cleaner No. 505, was known. The concentration of citric acid in the solution was 530 g/l.

Results

The patient experienced severe epigastric pain after ingestion. Physical examination findings before entering the ambulance were blood pressure 140 mmHg determined by palpitation, pulse rate 88 beats/min with regular rythm and respiratory rate of 40 breaths/min. Within an hour, his condition deteriorated; he was ashen, blood pressure was 80/40 mmHg and pulse rate was 102 beats/min. His neck vessels were flat, and breath sounds were equal bilaterally with occasional expiratory wheezes heard at both bases. There were no cardiac murmurs. The abdomen was short and bowel sounds were active. His extremities were warm with no cyanosis or oedema.

No abnormalities were detected on neurological examination.

Initial laboratory examination revealed a severe degree of metabolic acidosis (plasma pH 7.03, bicarbonate 12 mmol/l) and increased anion gap (32 mEq/l), hyperkalemia (6.3 mmol/l) and a normal plasma osmolal gap (see table below).

Table 10 : Laboratory examination

Characteristic	Admission	12 h After Admission
pH	7.03	7.40
Pco_2 (mm Hg)	35	38
Po_2 (mm Hg)	190*	129†
HCO_3^- (mEq/L)	12	23
Na^+ (mEq/L)	143	137
K^+ (mEq/L)	6.3	3.8
Cl^- (mEq/L)	99	103
Anion gap (mEq/L)	32	11
Osmolality (mOsm/kg H_2O)	291	—
Albumin (g/dL)	5.1	—
Glucose (mg/dL)	180	—
Blood urea nitrogen (mg/dL)	8	13
Creatinine (mg/dL)	1.4	0.9
Calcium (mg/dL)	10.2	7.5
Citrate (mEq/L)	20	<1
L-lactate (mEq/L)	2.7	—

Serum ketone and toxicologic screen results were both negative.
*Six liters of nasal oxygen.
†Two liters of nasal oxygen.

Total calcium was 10.2 mg/dl (normal range 8.8 – 10.3 mg/dl). The ECG showed sinus tachycardia and peaked T waves and the QT corrected interval was 453 ms.

A Foley catheter was inserted and 500 ml of blue-coloured urine was obtained. Urinalysis revealed occasional granular casts and frequent amorphous urate crystals but no calcium oxalate crystals were observed by means of polarising microscopy.

The patient received 1 L of Ringer's solution in the first hour but there was no change in blood pressure or heart rate.

Following administration of 1 g of calcium chloride, 50 mmol of sodium bicarbonate, 25 g of glucose and 10 units of regular insulin intravenously, the patient's blood pressure increased to 116/76 mmHg and pulse rate decreased to 90 beats/min. By the next morning plasma acid-base parameters were within normal range as was the ionised calcium level of 4.4 mg/dl (normal range 4.40 to 5.40 mg/dl). Subsequent evaluation of stored samples revealed a markedly elevated citrate level on admission (20 mEq/l).

Because of the short duration and severity of the anion gap type of metabolic acidosis together with a near normal L-lactate level, acid ingestion was the most likely basis for the acid-base disorder. This diagnosis was established once the composition of the ingested fluid was known.

Summary and conclusion

Following administration of 1 L of Ringer's solution in the first hour, 1 g of calcium chloride, 50 mmol of sodium bicarbonate, 25 g of glucose and 10 units of regular insulin intravenously, the patient's blood pressure increased to 116/76 mmHg and pulse rate decreased to 90 beats/min. By the next morning plasma acid-base parameters were within normal range as was the ionised calcium level of 4.4 mg/dl (normal range 4.40 to 5.40 mg/dl). Subsequent evaluation of stored samples revealed a markedly elevated citrate level on admission (20 mEq/l).

Although ionised calcium levels were not obtained early in this case, the rapid haemodynamic improvement with calcium chloride administration suggested that this patient had life-threatening low ionised calcium levels on presentation. This case also illustrates the need to consider the biological properties of both the H^+ and the conjugate base when assessing the dangers associated with metabolic acidosis of the anion gap type. Acute toxicity was probably caused by chelation of calcium by means of trivalent citrate. When citric acidosis is suspected, ionised Ca^{2+} administration can be a critical lifesaving component of therapy.

3.1.3 Other data

No other data available

3.2 Acute toxicity - dermal route

3.2.1 Animal data

3.2.1.1 [redacted] (2006)

Study reference:

[redacted] *(2006) Citric Acid: Acute Dermal Toxicity (Limit Test) in the Rat. SPL Project Number:* [redacted]

Detailed study summary and results:

OECD 402 and Method B.3 of Commission Directive 92/69/EEC

5 males and 5 females were housed individually during the 24-hr exposure period. Free access to drinking water and food. The temperature and relative humidity were set to achieve limits of 19 to 25 °C and 30 to 70 %, respectively,

All animals were dosed at 2000 mg/kg bw by applying with distilled water to an area of shorn skin (approximately 10 % of the total body surface area). The treatment area was semi-occluded with a bandage.

Exposure was for 24 hours and the treated area was wiped with cotton wool moistened with distilled water.

The animals were observed for deaths or overt signs of toxicity ½, 1, 2 and 4 hours after dosing and subsequently daily for 14 days.

Test sites were examined according to the Draize (1977) Dermal and Eye Toxicity Tests.

Test material	Citric acid
Lot/Batch number	S50423
Description	White crystalline solid
Purity	100%
Stability	Stable when kept at room temperature in the dark
Test Animals	
Species	Rat
Strain	Sprague-Dawley Cd (Crl:CD(SD) IGS BR)
Source	Charles River (UK)
Sex	Male and Female
Age/weight at study initiation	8-12 weeks old and at least 200 g
Number of animals per group	5/sex/group
Control animals	No
Postexposure period	0.5, 1, 2 and 4 hours and once daily for 14 days.
Area covered	10 % of body surface

Occlusion	Semi-occlusive
Vehicle	No
Concentration in vehicle	2000 mg/kg
Total volume applied	2000 mg/kg
Duration of exposure	24 h
Removal of test substance	Water
Controls	none

Reliability: 1

Results

There were no deaths.

There were no signs of systemic toxicity or dermal irritation.

All animals showed expected gains in bodyweight over the study period.

No abnormalities were noted at necropsy.

Table 11: Table for acute dermal toxicity

Dose [mg/kg bw]	*Number of dead / number of investigated*	*Time of death (range)*	*Observations*
2000	0/10		No signs of systemic toxicity, dermal irritation. No abnormalities noted at necropsy
LD_{50} value	>2000 mg/kg bw		

Conclusion

The acute dermal median lethal dose (LD_{50}) of the test material in the Sprague-Dawley CD strain rat was found to be greater than 2000 mg/kg bodyweight.

3.2.2 Human data

No human data available

3.2.3 Other data

No other data available

3.3 Acute toxicity - inhalation route

3.3.1 Animal data

No reliable studies available

3.3.2 Human data

No reliable studies available

3.3.3 Other data

No other data available

3.4 Skin corrosion/irritation

3.4.1 Animal data

3.4.1.1 REACH Registration dossier (2010)

Study reference:

REACH Registration dossier (2010) (study report 1990)

Detailed study summary and results:

Study performed in 6 rabbits, following OECD guideline 404

Type of coverage : semi-occlusive

Duration of exposure : 4 hours

Observation period : 0, 1, 24, 48 and 72 hours

Test material : 0.5 g powder

Vehicle : covered with path moistened with water

Result :

Well defined erythema in 1/6 from 1 - 48 h; mild erythema in the same animal was still evident at 72 h when study was terminated. See table:

Score at time point / Reversibility	**Erythema/ eschar (max score: 4)**	**Edema (max score: 4)**
1 h	2/0/0/0/0/0	0/0/0/0/0/0
24 h	2/0/0/0/0/0	1/0/0/0/0/0
48 h	2/0/0/0/0/0	0/0/0/0/0/0
72 h	1/0/0/0/0/0	0/0/0/0/0/0
Average given in report: 1h, 24h, 48h, 72h	0.3	0
Average - EU criteria: 24h, 48h, 72h	0.3	0
Reversibility*	c	n/a
Average time for reversion	unclear (likely to be reversible)	
* Reversibility: c. = completely reversible; n.c. = not completely reversible; n. = not reversible Under the conditions of this test, citric acid is not irritating to the skin of rabbits		

Reliability: 1

3.4.1.2 REACH Registration dossier (2010)

Study reference:

REACH Registration dossier (2010) (study report 1990)

Detailed study summary and results:

Study performed in 6 rabbits, following OECD guideline 404

Type of coverage : semi-occlusive

Duration of exposure : 4 hours

Observation period : 0, 1, 24, 48 and 72 hours

Test material : Dose not stated

Vehicle : covered with path moistened with water

Result :

There were no changes in the untreated adjacent skin (control) of any rabbit at any time interval. Well defined erythema was observed in 1/6 animals within 30 - 60 minutes post-exposure and again at 24 and 48 hours. By 72 hours postexposure, erythema was slight, indicating that the skin was recovering and returning to normal.

PDII (72h) = 0.33
According to Draize criteria, citric acid was slightly irritating in this study, but it does not require classification according to EU criteria

Reliability: 1

3.4.1.3 REACH Registration dossier (2010)

Study reference:

REACH Registration dossier (2010) (study report 1979)

Detailed study summary and results:

Study performed in 6 rabbits.

Type of coverage : occlusive

Duration of exposure : 4 hours

Observation period : 0 and 44 hours

Vehicle : water

Result :

Overall irritation score : 0 at 0 and 44 hours (no maximum score given)

Conclusion :
Not irritating

Reliability: 2

3.4.1.4 REACH Registration dossier (2010)

Study reference:

REACH Registration dossier (2010) (study report 1984)

Detailed study summary and results:

Study performed in 3 rabbits, following OECD guideline 404

Type of coverage : occlusive

Duration of exposure : 4 hours

Observation period : 0, 20 and 44 hours

Vehicle : water

Result :

PDII : 0.8/8 (not fully reversible within 44 hours)

Table 12 : Summary of erythema and edema examinations

	Erythema (maximum score 4)	Edema (maximum score 4)
Intact skin :		
0 hours	0/0/0	0/0/0
20 hours	0/0/0	0/0/0
44 hours	0/0/0	0/0/0
Abraded skin :		
0 hour	1/1/2	1/1/1
20 hours	1/1/1	0/0/1
44 hours	1/1/1	0/0/1

Conclusion :
Not irritating

Reliability: 2

3.4.1.5 ECB (2000)

Study reference:

ECB (2000) IUCLID Dataset Citric Acid.

Detailed study summary and results:

Test type

OECD 404 'Acute Dermal Irritation/Corrosion'

Test substance

- Citric acid; no details on purity

Test animals

- Rabbit
- 6 animals per per dose
- Age/weight: Not stated

Administration/exposure

- Duration of test/exposure period: Not stated
- Total dose: amount/concentration of test material applied to skin in mg/ml and rationale for dose level selection: Not stated
- Post exposure observation period: 72 hours
- Control group and treatment: not stated
- Vehicle: identification, concentration and volume used, justification of choice of vehicle (if other than water): not stated
- Time points at which grading/scoring took place: 24, 48, 72 hours
- Grading scale: not stated
- Preparation of the test site, etc: not stated
- Removal of test substance (e.g. water or solvent): not stated
- Statistical methods: not stated

Results and discussion

- Average result from test on 6 rabbits after 24, 48 and 72 hours: Erythema = 0; Oedema = 0

Reliability: 4

3.4.1.6 ECB (2000)

Study reference:

ECB (2000) IUCLID Dataset Citric Acid.

Detailed study summary and results:

Test type

OECD 404 'Acute Dermal Irritation/Corrosion'

Test substance

- Citric acid; no details on purity

Test animals

- Rabbit
- Animals per per dose: not stated

Administration/exposure

- Duration of test/exposure period: Not stated
- Total dose: amount/concentration of test material applied to skin in mg/ml and rationale for dose level selection: Not stated
- Post exposure observation period: 72 hours
- Control group and treatment: not stated
- Vehicle: identification, concentration and volume used, justification of choice of vehicle (if other than water): not stated
- Time points at which grading/scoring took place: 24, 48, 72 hours
- Grading scale: not stated
- Preparation of the test site, etc: not stated
- Removal of test substance (e.g. water or solvent): not stated
- Statistical methods: not stated

Results and discussion

- Average result from test on 6 rabbits after 24, 48 and 72 hours: Erythema = 0.33; Oedema = 0
- Described as slightly irritating
- Reliability: 4

3.4.1.7 OECD SIDS (2001)

Study reference:

OECD SIDS (2001) Full SIDS Summary Citric Acid.

Detailed study summary and results:

Severe ulceration and tissue damage occured in dogs receiving tongue application of 0.1 ml of 50% citric acid solution for 5 minute

No further information is available from the SIDS dossier.

3.4.2 Human data

3.4.2.1 [Study 1]

Study reference:

Surveys by the USA FDA, Yates et al., 1999; Barrows, 2002

Detailed study summary and results:

A survey by the USA FDA identified that AHA products (alpha hydroxyl acids) were available to various levels of practitioner based on the concentration of AHA:

General public use: 10% or less;

Trained cosmetologists: 20 – 30%;

Doctors: 50 – 70%.

Citric acid has been identified as one of a group of ingredients collectively known as alpha hydroxyl acids or AHAs. AHAs were originally used by doctors as chemical peels. While the precise mechanism of action of the AHA is not known, the acid irritates the skin so triggering a healing response and cell renewal.

This demonstrates the irritant potential of citric acid at the above concentrations.

3.4.3 Other data

No other data available.

3.5 Serious eye damage/eye irritation

3.5.1 Animal data

3.5.1.1 [redacted] (1984)

Study reference:

[redacted] *(1984), Determination of Acute Eye Irritation on rabbits with Ro01-7548 – citric acid powdered – 014 3271 7, research report No. B-105'402.*

Detailed study summary and results:

The effects of Citric Acid on eyes were examined in rabbits according to International Regulatory Liaison Group and OECD 405.

A 10 % aqueous solution of citric acid induced shortlasting conjunctival irritation only (reversible after 1 week), while a 30 % aqueous solution of citric acid caused well-defined to moderate conjunctival irritation of the rabbit eye accompanied by discharge and superficial ulceration of conjunctival epithelium.

The results of this study indicate that Citric Acid at concentration levels above 30 % may induce moderate to strong conjunctival irritation accompanied by superficial lesion of conjunctival epithelium.

Test material	Citric Acid
Lot/Batch number	Ro 1-7548
Specification	Not specified
Description	Powder
Purity	Not specified
Stability	Not specified
Test Animals	
Species	Rabbit
Strain	New Zealand white
Source	Füllins-dorf Breeding Farm
Sex	Male and female
Age/weight at study initiation	Age: adult Weight: 2 kg

Number of animals per group	3
Control animals	Yes. Concurrent no treatment
Administration/ Exposure	
Preparation of test substance	Test substance was used as delivered
Amount of active substance instilled	0.1 mL
Exposure period	Single administration
Postexposure period	14 days
Examinations	
Ophthalmoscopic examination	Yes. Examination of reactions was performed by naked eye or was facilitated by use of a loupe. After recording the observations at 24 hours, the eyes of any or all rabbits may be further examined with the aid of fluorescin.
Scoring system	Draize
Examination time points	Clinical observation and scoring: The eyes were examined at 1, 24, 48 and 72 hours. If there were no evidence of irritation at 72 hours the study was ended. In addition observation of the cornea, iris and conjunctivae, and any other lesions were noted up to 14 days.

Reliability: 1

Results

None of the rabbits died during the study period. All control eyes were free of ocular irritation and other findings at all observations.

Reversibility: Moderate to weak conjunctival irritation of the rabbit eye caused by 10 % aqueous solution, disappeared within 1 week.

Table 13 : Primary eye irritation score (Draize)

Test Conc.	Animal No.	Positive (+) or Negative (-) Scores					Clear at Day	Maximum Draize Score
		Cornea	Iris	Conjunctiva				
				Redness	Chemosis	Ulceration		
10 %	1	-	-	+	+	-	7	10
	2	-	-	+	+	-	7	8
	3	-	-	+	+	-	7	10
	Total	-	-	3	3	-		28
								Mean : 9.3
30 %	1	-	-	+	+	+	14	16
	2	-	-	+	+	+	Not clear	16
	3	-	-	+	+	+	14	16

	Total	-	-	3	3	3		48
								Mean : 16.0

Table 14 : summary of eye irritation score : test with 10% aqueous solution

	Cornea			Iris (max score of 2)	Conjunctivae			Discharge (max score of 3)
Score at time point	Opacity (max score of 4)	Ulceration	Positive stain retention		Redness (max score of 3)	Chemosis (max score of 4)	Ulceration	
1 h	0/0/0	0/0/0	0/0/0	0/0/0	2/2/2	2/1/2	0/0/0	1/1/1
24 h	0/0/0	0/0/0	0/0/0	0/0/0	1/1/1	0/0/0	0/0/0	0/0/0
48 h	0/0/0	0/0/0	0/0/0	0/0/0	1/1/1	0/0/0	0/0/0	0/0/0
72 h	0/0/0	0/0/0	0/0/0	0/0/0	1/1/1	0/0/0	0/0/0	0/0/0
7 days	0/0/0	0/0/0	0/0/0	0/0/0	0/0/0	0/0/0	0/0/0	0/0/0
14 days	0/0/0	0/0/0	0/0/0	0/0/0	0/0/0	0/0/0	0/0/0	0/0/0
Average at 24, 48 and 72 h	0/0/0	-	-	0/0/0	1/1/1	0/0/0	0/0/0	0/0/0
Draize maximum average score	0	-	-	0	9.3			
Reversibility	-	-	-	-	C	C	-	C
Time for reversion					7 days	24 h		24 h

Reversibility : c = completely reversible

Table 15 : summary of eye irritation score : test with 30 % aqueous solution

	Cornea			Iris (max score of 2)	Conjunctivae			Discharge (max score of 3)
Score at time point	Opacity (max score of 4)	Ulceration	Positive stain retention		Redness (max score of 3)	Chemosis (max score of 4)	Ulceration (u)	
1 h	0/0/0	0/0/0	0/0/0	0/0/0	3/3/3	3/3/3	0/0/0	2/2/2

24 h	0/0/0	0/0/0	0/0/0	0/0/0	3/3/3	3/3/3	u/u/u	2/2/2
48 h	0/0/0	0/0/0	0/0/0	0/0/0	3/3/3	2/3/2	u/u/u	2/2/2
72 h	0/0/0	0/0/0	0/0/0	0/0/0	3/3/3	2/2/2	u/u/u	2/2/2
7 days	0/0/0	0/0/0	0/0/0	0/0/0	1/1/1	1/1/1	0/0/0	0/0/0
14 days	0/0/0	0/0/0	0/0/0	0/0/0	0/1/0	0/0/0	0/0/0	0/0/0
Average at 24, 48 and 72 h	0/0/0	-	-	0/0/0	3/3/3	2.3/2.7/2.3	0/0/0	2/2/2
Draize maximum average score	0	-	-	0	16			
Reversibility	-	-	-	-	N.C	C	-	C
Time for reversion					By 14 days	By 14 days		By 14 days

Reversibility : c = completely reversible, N.C : not completely reversible

3.5.2 Human data

No relevant data available.

3.5.3 Other data

No relevant data available.

3.6 Respiratory sensitisation

3.6.1 Animal data

No data available

3.6.2 Human data

3.6.2.1 Barros M.J. et al. (1990)

Study reference:

Barros M.J. et al.(1990), Importance of inspiratory flow rate in the cough response to citric acid inhalation in normal subjects, Clinical Science, 78 (5), p 521-525.

Detailed study summary and results:

11 non-smoking, healthy volunteers received doubling concentrations of citric acid, at 3 different IFRs (inspiratory flow rates). These were given on 3 days, at the same time of the day and at least 48 h apart. The order of the 3 IFRs was randomised. 3 inhalations at each concentration, from residual volume to total lung capacity, were given 1 min apart. The starting concentration was 2.5 mg/l and the maximum 640 mg/l. There was a 3-min interval between concentrations and the test finished when a cough was produced at each

inhalation (cough threshold) or the maximum concentration was reached. All coughs up to 30 s after the beginning of each inhalation were registered.

Substance	Citric acid
Persons exposed	
Sex	9 males and 2 females
Age/weight	Age: 23 – 39 years Weight: Not specified
Known Diseases	Healthy
Number of persons	11
Other information	The subjects were all non-smokers.
Exposure	Inhalation
Reason of exposure	Medical testing
Frequency of exposure	Multiple The subjects received doubling concentrations of citric acid at 3 different IFR.
Overall time period of exposure	The subjects received doubling concentrations of citric acid at 3 different IFR. These were given on 3 days, at the same time of the day and at least 48 h apart.
Duration of single exposure	The 3 inhalations were given 1 min apart. There was a 3-min interval between concentrations and the test finished when a cough was produced at each inhalation (cough threshold) or the maximum concentration was reached.
Exposure concentration/dose	Amounts of drug delivered were calculated by adding the amount in the internal volume of the apparatus at the beginning of each inhalation and the output of the nebuliser during additional inspiratory time.
Examinations	Volume inspired during the first second, total volume inspired, peak inspiratory flow and flow at the end of the first second of inspiration. The inspiratory manoeuvres and cough were recorded with a respiratory inductance plethysmograph on a Mingograf 803 ink jet recorder. The latency period (time between the start of the inspiration and the first cough) was obtained in order to calculate the cough index (number of coughs divided by the latency). Forced expiratory volume in 1 s (FEV1), functional vital capacity (FVC) and peak expiratory flow rate (PEFR) were measured before and after each complete challenge.

Results

Clinical signs: No relevant effects, other than cough were observed.

Baseline FEV1, FVC and PEFR were not different on the 3 study days. No significant changes were seen after citric acid inhalations in any of these tests. Cough thresholds varied between subjects. Thresholds were increased at higher IFRs in 5 subjects, with the cough threshold at 150 l/min significantly higher than that at 50 l/min for the group.

The cough threshold was 21 mg/l at an inspiratory flow rate of 50 l/min and 43 mg/l at 150 l/min. The amount of drug tolerated by the subjects before the cough threshold was achieved was 5.2 mg at an

inspiratory flow rate of 50 l/min and 11.6 mg at 150 l/min. The number of coughs per inhalation was 1.6 at an inspiratory flow rate of 50 l/min and 1.1 at 150 l/min.

Conclusion

Lower inspiratory rates were associated with a greater cough stimulus in the citric acid challenge procedure used in this study. This may be related to increased laryngeal deposition.

3.6.2.2 Winther F.O. (1970)

Study reference:

Winther F.O. (1970), Experimentally induced cough in man by citric acid aerosol. An evaluation of a method. Acta pharmocol. et toxicol., Vol. 28, ISS 2, p 108-112.

Detailed study summary and results:

Substance	Citric acid aerosol
Persons exposed	
Sex	Males
Age/weight	Age: 20 – 50 years
	Weight: Not specified
Known Diseases	Healthy, with no disease of the respiratory tract
Number of persons	10
Other information	None of the subjects were heavy smokers (less than 10 cigarettes per day)
Exposure	Inhalation
Reason of exposure	Medical testing
Frequency of exposure	Multiple
	The subjects underwent 3 test periods, with each test period consisting of 5 inhalations.
Overall time period of exposure	Each of the 3 test periods consisted of 5 inhalations with at least a 3-minute interval. There was an interval of 1 hour between test periods.
Duration of single exposure	Not specified
Exposure concentration/dose	Measured
Other information	
Examinations	After each inhalation the number of coughs was counted.

The citric acid solution was nebulised in an air flow of 8 l/min. Each subject had a training period. During this period the concentration of nebulised citric acid solution which could produce between 3 and 6 coughs after one single inhalation was determined. The concentration ranged from 5 to 25 %:

No. of persons	Concentration of citric acid solution
3	5 %

2	10 %
3	15 %
1	20 %
1	25 %

Placebo tablets were administered immediately after the first test period. The subjects tested were informed that they would be given either an antitussive tablet or a placebo tablet. Each of the 3 test periods consisted of 5 inhalations with at least a 3-minute interval. After each inhalation the number of coughs was counted. As soon as the cough had stopped the subject tested was given a few sips of lukewarm water so as to clear the mouth and the throat of citric acid.

Results

Clinical signs: No relevant effects, other than cough, were observed.

There is a moderate reduction in the number of coughs from one test period to the next and a marked fall within each test period. The latter fall, however is only seen between the first and the second inspiration.

Summary and conclusion

Initially the reaction was very inconsistent but after 5 to 8 inspirations the number of coughs became stabilised at an apparently constant level at which we started the registrations. There were 3 test periods with an interval of 1 h. Each test period was preceded by about 5 inhalations which were not taken into account.

After initial adaptation during the training period, further adaptation to citric acid aerosol appeared to be small taking into account the whole test periods. The decrease between the first and second test period might be ascribed to a placebo reaction. Within each test period, however, a clearly significant reduction from the first inspiration to the following four inspirations occurred. It is regarded that this reduction is due to an adaptation similar to that seen in the training period.

3.6.2.3 Empey D.W. et al. (1976)

Study reference:

Empey D.W. et al. (1976), Mechanisms of bronchial hyperactivity in normal subjects after upper respiratory tract infection, American Review of Respiratory Disease, Volume 113 (2), p 131-139, 1976.

Detailed study summary and results:

16 otherwise healthy volunteers who had uncomplicated upper respiratory tract infections (colds) were used, 12 were studied in detail during and after their colds. A group of healthy control subjects was drawn from the same population.

Fresh solutions of chemicals were prepared daily. Normal saline, buffered to pH 7 with sodium bicarbonate was used as a control solution. Citric acid solution (20 % in distilled water) was diluted with normal saline to concentrations ranging from 0.25 – 20 %. All solutions were delivered as aerosols.

Six subjects with colds and 6 control subjects inhaled 5 breaths of 10 % citric acid. Measurements of airway resistance were made immediately after inhalation and again every 10 sec for 3 min. To examine the effects of citric acid on cough, 7 subjects with colds and 12 control subjects using a modification of the method described by Bickerman and Barach. The subjects were seated in a quiet room and told to breathe out slowly to residual volume and then to inhale the aerosol (saline or 0.25 – 20 % citric acid) rapidly until they reached total lung capacity.

Substance	Histamine diphosphate aerosol and citric acid aerosol (only citric acid is discussed in the summary).
Persons exposed	
Sex	Male and female

Age/weight	Subjects' ages: 22 – 37 years Controls' ages: 23 – 36 years Weight: Not specified
Known Diseases	The subjects had uncomplicated upper respiratory tract infections (colds) but were otherwise healthy. None of the control group had had a cold during the 2 months preceding the study.
Number of persons	16 (12 were studied in detail during and after their colds) in the study group and 11 in the control group.
Other information	Study group: 3 persons smoked 10 – 20 cigarettes per day and 2 had a history of hay fever but had no symptoms at the time of the study. None had a personal or family history of asthma and none had suffered from any lung diseases. General physical examination was normal. Control group: 2 smoked 10 – 20 cigarettes per day and 2 had a history of hay fever.
Exposure	Inhalation
Reason of exposure	Medical testing
Frequency of exposure	Multiple When studying the effects of citric acid on cough, there was a 1-min interval after each inhalation.
Overall time period of exposure	Not specified
Duration of single exposure	6 subjects with colds inhaled 5 breaths of 10 % citric acid. For the effects of citric acid on cough the subjects inhaled the aerosol rapidly until they reached total lung capacity.
Exposure concentration/dose	Measured

Examinations	6 subjects with colds and 6 control subjects inhaled 5 breaths of 10 % citric acid. Measurements of airway resistance were made immediately after inhalation and again every 10 sec for 3 min. The changes at each 10 sec period after inhalation of citric acid were compared to the baseline values. Subjects were studied again 6 weeks after recovery. To examine the effects of citric acid on cough, 7 subjects with colds and 12 control subjects were seated in a quiet room and told to breathe out slowly to residual volume and then to inhale the aerosol (saline or 0.25 – 20 % citric acid) rapidly until they reached total lung capacity. The number of coughs after this manoeuvre was recorded and the lowest concentration of citric acid that reproducibly (3 times) elicited more than 2 involuntary coughs was considered to be a threshold dose. Weaker concentrations of citric acid were always used first. There was a 1-min interval after each inhalation.

Results

In six subjects with colds, citric acid aerosol (10 %, 5 breaths) caused bronchoconstriction that lasted up to 30 sec after inhalation, a significantly greater effect than that observed in control subjects or in the same subjects after recovery. Prior inhalation of atropine aerosol (0.2 %, 20 breaths) prevented the bronchoconstriction after citric acid aerosol in all six subjects.

The threshold concentration of citric acid that produced cough in seven subjects with colds (median 2 %) was significantly lower than that in control subjects (median 3.75 %) or in the seven subjects after recovery, suggesting the exaggerated cholinergic response was due to a decreased threshold doe stimulation of the rapidly adapting sensory receptors in the airways.

Conclusion

The study demonstrates that spontaneous, presumably viral, upper respiratory tract infections cause striking increases in bronchial reactivity to inhaled histamine and citric acid aerosols in otherwise normal subjects

3.6.3 Other data

No data available.

3.7 Skin sensitisation

3.7.1 Animal data

No data available.

3.7.2 Human data

3.7.2.1 [redacted] (2002)

Study reference:

[redacted] *(2002), The occlusive repeated insult patch study in humans. TKL Research, USA KC. Study No. 2416 (.05)*

Detailed study summary and results:

In a study performed in accordance with GCP standards, 209 human subjects were treated with a 2 cm x 2 cm occlusive patch of the biocidal product applied topically for 9 consecutive days. Patches were removed 24 hours after application and observations recorded

After a rest period of 10 – 15 days, one patch was applied on previously unexposed skin. The patch was removed after 24 hours and observations recorded at 24 and 48 hours after removal.

Test material	*Test Code No. 2416.05*
Lot/Batch number	*Test Code No. 2416.05*
Purity	*99.5%*
Stability	*Stable*
Preparation of test substance for application	*Product used as marketed.*
Pretest performed on irritant effects	No
Test Animals	Non-entry field
Species	*Human*
Strain	*Not applicable*
Source	*Not applicable*
Sex	*Male/female*
Age/weight at study initiation	*Age 18 and above*
Number of animals per group	*209 subjects (197 completed the study)*
Control animals	No
Administration/ Exposure	*Non-Adjuvant*
Induction schedule	*9 consecutive applications of study material (24-hour exposure period)*
Way of Induction	topical
	Occlusive
Concentrations used for induction	2 cm x 2 cm patch of tissue
Concentration Freunds Complete Adjuvant (FCA)	Not applicable
Challenge schedule	Challenge initiated during 6th week of study (10 - 15 rest days between induction and challenge).
Concentrations used for challenge	2 cm x 2cm patch of tissue applied to previously unexposed skin.
Rechallenge	No
Scoring schedule	24h, 48h after challenge
Removal of the test substance	Patches were removed 24 hours after application

Positive control substance None

Results

174/197 subjects elicited no response during the challenge

22/197 had a minimal or doubtful response

1/197 showed definite erythema only

Conclusion

Under the conditions of the study the substance is not sensitising.

3.7.3 Other data

No data available.

3.8 Germ cell mutagenicity

3.8.1 *In vitro* data

3.8.1.1 Ishidate Jr M. et al. (1984)

Study reference:

Ishidate Jr. M. et al. (1984), Primary Mutagenicity Screening of Food Additives Currently Used in Japan, Food and Chemical Toxicology, Vol. 22, No. 8, PP. 623-636

Detailed study summary and results:

Citric acid purity = 99.9%

In a study performed using a method similar to OECD 471, citric acid was tested for mutagenic effects in Salmonella thyphimurium TA1535, TA100, TA98, TA1537, TA92, TA94 with and without metabolic activation (liver homogenate from rats pretreated with polychlorinated biphenyl KC-400). The report does not give details of what concentrations of citric acid were used except that it was up to 5mg/plate.

No mutagenic effects were reported in the test, i.e. no increased incidence of revertant colonies at maximum plate concentration (5m/plate)

Based on the results of this study citric acid is considered non-mutagenic.

3.8.1.2 Ishidate Jr M. et al. (1984)

Study reference:

Ishidate Jr. M. et al. (1984), Primary Mutagenicity Screening of Food Additives Currently Used in Japan, Food and Chemical Toxicology, Vol. 22, No. 8, PP. 623-636

Detailed study summary and results:

Citric acid purity = 99.9%

No guidelines were given followed in this study, but method is similar to OECD 473.

Chinese hamster fibroblasts were exposed to three different doses of which only the maximum dose is reported. The maximum dose was 1 mg/ml.

Test material	Citric acid, granular, U.S.P Control Number 121M231 IV, as supplied by the Food and Drug Administration
Lot/Batch number	No data
Specification	No data
Description	No data
Purity	99.9%
Stability	No data
Study Type	*In vitro* mammalian chromosome aberration test
Organism/cell type	Chinese hamster fibroblast cell line (CHL), from the lung of a newborn female
Deficiencies / Proficiencies	n/a
Metabolic activation system	None applied
Positive control	No data
Administration / Exposure; Application of test substance	Non-entry field
Concentrations	Up to 1 mg/ml
Way of application	Spread on clean glass slides , solvent was physiological saline
Pre-incubation time	No data.
Other modifications	No data

Chromosome preparation were made as follows. Colcemid (final conc. 0.2µg/ml) was added to the culture 2 hrs before cell harvesting. The cells were then trypsinised and suspended in a hypotonic KCL solution (0.075M) for 13 min at room temperature. After centrifugation the cells were fixed with acetic acid-methanol (1:3, v/v) and spread on clean glass slides. After air-drying the slides were stained with Giemsa solution (1.5% at pH 6.8) for 12-15 mins. A hundred well spaced metaphase were observed under the microscope.

Results

Purity (%)	Max Dose (mg/ml)	Polyploid (%)	Struct. Aber. § (%)	Struct. Aber. § (hr)	Result
99.9	1	0.0	0.0	48	Negative

No structural aberrations were observed after 48 hours.

Conclusion

Based on the results of this study citric acid is considered non-clastogenic.

3.8.1.3 [redacted] (1975)

Study reference:

[redacted] *(1975), Summary of Mutagenicity Screening Studies: Host Mediated Assay Cytogenetics, Dominant Lethal Assay. Contract FDA 71-268, Compound FDA 71-54 Citric Acid. Litton Bionetics, Inc*

Detailed study summary and results:

Human embryonic lung cultures (WI-38) which were negative for adventitious agents (viruses, mycoplasma) which may interfere were used. These cells were employed at passage level 19. The cells had been transferred using 0.025 % trypsin and planted in 32 oz. prescription bottles containing 40 ml tissue culture medium. When growth was approximately 95 % confluent the cells were removed from the glass using trypsin, centrifuged, and frozen in tissue culture medium containing dimethyl sulfoxide (DMSO). Cells were frozen in vials in the vapour phase of liquid nitrogen at a concentration of 2 x 10^6 cells/ml. When needed, the vials were removed from liquid nitrogen, quick thawed in a 37 °C water bath, washed, suspended in tissue culture medium (minimal essential medium plus 1 % glutamine, 200 units/ml of penicllin and 200 µg/ml of streptomycin and 15 % fetal calf serum) and planted in milk dilution bottles at a concentration of 5 x 10^5 cells/ml. The test compound was added at three dose levels usiong three bottles for each level, 24 hours after planting. The dose levels required a preliminary determination of a tissue culture toxicity. This was accomplished by adding logarithmic doses of the compound in saline to a series of tubes containing 5 x 10^5 cells/ml which were almost confluent. The cells were examined at 24, 48, 72 hours. Any cytopathic effect (CPE) or inhibition of mitoses was scored as toxicity. Five more closely spaced dose levels were employed within the two logarithmic dosages, the higher of which showed toxicity and the lower no effect. The dose level below the lowest toxic level was employed as the high level. Logarithmic dose levels were employed for the medium and low levels.

Cells were incubated at 37 °C and examined twice daily to determine when an adequate number of mitoses were present. Cells were harvested by shaking when sufficient mitoses were observed, usually 24 - 48 hours after planting, centrifuged and fixed in absolute methanol:glacial acetic acid(3:1) for 30 minutes.

The specimens were centrifuged, decanted, and suspended in acetic acid-orcein stain (2.0 %) and a drop of suspension placed on a clean dry slide. Selected cover glasses 0.17 mm in thickness were placed on the suspension and the excess stain gently expressed from the slide. The cover glasses were sealed with the clear nail polish and examined immediately.

The microscopes, objectives, oculars, filters and light sources were enumerated under the metaphase description. Positive controls were triethylene melamine (TEM) at a concentration of 0.1 µg/ml dissolved in saline, and negative controls which consisted of the vehicle in which the test compound was dissolved, which was 0.85 % saline.

Note: dose levels were 6.0, 60, 600 µg/ml.

Test material	Citric acid
Lot/Batch number	U.S.P Control Number 121M231 IV
Specification	n/a
Description	Granular
Purity	n/a
Stability	n/a
Study Type	Cytogenetic assay.
Organism/cell type	Human embryonic lung cultures (WI-38)
Deficiencies / Proficiencies	Negative for adventitious agents (viruses, mycoplasm) which may interfere.
Metabolic activation system	None
Positive control	Triethylene Melamine (0.1 µg/ml) dissolved in saline.
Negative control	Vehicle which test compound was dissolved in (0.85 % saline).

Administration / Exposure; Application of test substance	Non-entry field
Concentrations	6.0, 60, 600 µg/ml
Way of application	Dissolved in medium
Pre-incubation time	24 hours after planting
Other modifications	n/a
Examinations	
Number of cells evaluated	100 cells per dose level.

Results

The negative control contained two cells with bridges. The low dosage level of the test compound contained one cell with a bridge and the high level contained two cells with bridges. The positive control was within normal values.

Conclusion

Citric acid produced no detectable significant aberration in the anaphase chromosome of human tissue culture cells when tested at the dosage levels employed in this study.

3.8.1.4 Yilmaz S. et al. (2008)

Study reference:

Yilmaz S. et al. (2008), Clastogenic effects of food additive citric acid in human peripheral lymphocytes, Cytotechnology 56, 137-144

Detailed study summary and results:

Materials and methods

Test guideline: equivalent or similar to OECD Guideline 473 (*In vitro* Mammalian Chromosome Aberration Test)

Deviations: no activation, aberrations scored included sister chromatid unions

GLP compliance: no data

Type of genotoxicity: chromosome aberration

Type of study: *in vitro* mammalian chromosome aberration test

Test materials

Details on test material: Name of test material (as cited in study report): citric acid

Method

Species / strain / cell line

Species / strain / cell line: lymphocytes: peripheral human

Metabolic activation: without

Test concentrations: 50, 100, 200, 3000 µg/ml

Vehicle:

Vehicle(s)/solvent(s) used: water

Justification for choice of solvent/vehicle: none given

Controls

Negative controls: no

Solvent / vehicle controls: yes

Positive controls: yes

Positive control substance: cyclophosphamide

Details on test system and conditions:

METHOD OF APPLICATION: in medium

DURATION
- Exposure duration: 24 and 48 hours

- Expression time (cells in growth medium): 24 and 48 hours

- Fixation time (start of exposure up to fixation or harvest of cells): 24 and 48 hours

SPINDLE INHIBITOR (cytogenetic assays): colchicine added 2h before harvesting

STAIN (for cytogenetic assays): Giesma

NUMBER OF REPLICATIONS: duplicate cultures (two donors: healthy non-smokers, 27 years, 1 male, 1 female)

NUMBER OF CELLS EVALUATED: 100/donor (chromosome analysis), 1000/donor (mitotic index)

DETERMINATION OF CYTOTOXICITY

- Method: mitotic index

OTHER EXAMINATIONS:

- Determination of polyploidy: yes

- Determination of endoreplication: yes

- Other: cells were scored for Sister chromatid unions as well as chromatid and chromosome breaks, chromatid exchanges and dicentrics

Evaluation criteria: None given in report

Statistics: Statistical significance is referred to but no details are given.

Results and discussion

Test results

Species / strain / cell line: lymphocytes: peripheral human lymphocytes

Metabolic activation: without

Genotoxicity: positive

Remarks: including sister chromatid unions

Cytotoxicity: yes

Remarks: 3000 µg/ml

Vehicle controls valid: yes

Negative controls valid: not examined

Positive controls valid: yes

Additional information on results:

TEST-SPECIFIC CONFOUNDING FACTORS

- Effects of pH: reported "without changing the pH of the medium"

RANGE-FINDING/SCREENING STUDIES: not reported

COMPARISON WITH HISTORICAL CONTROL DATA: not compared with historical control data

Any other information on results incl. tables:

Table 16 Results of chromosome aberration analysis in cultured human lymphocytes

Test substance	Treatment	Dose	Structural Aberrations					Numerical Aberrations		Abnormal cell ± SE (%)	CA/Cell ± SE
	Period (hour)	(µg ml^{-1})	scu	ctb	dic	csb	cte	p	er		
Negative control	24	0.00	2	–	–	–	–	–	-	2.50 ± 0.70	0.025 ± 0.01
Positive control	24	0.10	18	25	7	3	12	3	–	30.00 ± 3.24	0.325 ± 0.03
Citric acid	24	50	15	4	5	1	–	1	1	12.50 ± 2.34*	0.135 ± 0.02*
		100	25	5	7	1	–	5	–	19.00 ± 2.77*	0.190 ± 0.03*
		200	35	5	10	–	–	5	–	20.00 ± 2.83*	0.250 ± 0.03*
		3,000	–	–	–	–	–	–	–	Toxic	Toxic
Negative control	48	0.00	3	1	–	–	–	–	–	2.00 ± 0.98	0.020 ± 0.01
Positive control	48	0.10	42	7	6	–	31	3	–	34.00 ± 3.35	0.430 ± 0.035
Citric acid	48	50	34	2	2	–	–	1	–	19.00 ± 2.77*	0.070 ± 0.03*
		100	27	9	–	–	1	1	–	18.50 ± 2.75*	0.100 ± 0.03*
		200	30	2	3	1	1	4	–	18.50 ± 2.75*	0.105 ± 0.03*
		3,000	–	–	–	–	–	–	–	Toxic	Toxic

* statistically significant ($p < 0.001$ (z-test)

Scu, Sister chromatid union; ctb, chromatid break; dic, dicentric; csb, chromosome break; cte, chromatid exchange; p, polyploidy; er, endoreduplication; CA/cell, (Chromosome aberrations/cell)

Applicant's summary and conclusion

Conclusions:

Citric acid has been tested according to a method that is similar to OECD 473, without activation. A statistically significant and dose-related increase in the number of cells with aberrations was observed after 24 and 48 hours incubation, including sister chromatid unions which are not usually reported. Insufficient information is provided to analyse the data excluding sister chromatid unions. It is concluded that there may be potential for citric acid to induce chromosome aberrations in cultured human lymphocytes under the conditions of this study.

Interpretation of results

Interpretation of results: positive without metabolic activation

3.8.2 Animal data

3.8.2.1 [redacted] (1975)

Study reference:

[redacted] *(1975), Summary of Mutagenicity Screening Studies: Host Mediated Assay Cytogenetics, Dominant Lethal Assay. Contract FDA 71-268, Compound FDA 71-54 Citric Acid. Litton Bionetics, Inc.*

Detailed study summary and results:

Ten to twelve week old, male rats were used and 15 animals per dose level were dosed by gastric intubation. To arrest the bone marrow cells in C-mitosis, 4 mg/kg of colcemid was administered intraperitoneally 2 hours prior to sacrifice.

The adhering muscle and epiphysis of one femur were removed and the marrow plug removed with a tuberculin syringe prior to aspiration into 5ml of Hanks balanced salt solution. The solution was centrifuged at 1500 RPM for 5 min, decanted and 2ml of hypotonic 0.5 KCl added with gentle agitation to resuspend the cells. In order to swell the cells, the samples were placed in a water bath at 37 ºC for 20 min.

The samples were then centrifuged for 5 min at 1500 RPM, decanted and a fixative (3:1 absolute methanol/ glacial acetic acid) added.

2/3 drops of the suspension were dropped onto a slide at 15º from horizontal and ignited. The slides were allowed to cool overnight and stained with a 5 % Giemsa solution.

The chromosomes of each cell were counted and only diploid cells were analysed. The cells were scored for chromatid gaps and breaks, chromosome gaps and breaks and reunions.

Test material	Citric acid
Lot/Batch number	U.S.P Control Number 121M231 IV
Test Animals	
Species	Rat
Strain	Sprague-Dawley
Source	Not stated
Sex	Male
Age/weight at study initiation	10 to 12 weeks old at time of use.
Number of animals per group	15 per dose level
Control animals	Yes
Administration/ Exposure	
Number of applications	1 (Acute); 5 (Subacute)
Post exposure period	6, 24, 48 h
	Oral
Type	Oral intubation

Concentration	Acute study: 500, 3500 mg/kg bw Subacute study: 300, 3000 mg/kg bw
Vehicle	Not stated
Concentration in vehicle	Not stated
Total volume applied	Not stated
Controls	Negative control, saline. Positive control, Triethylene melamine (TEM) 0.30 mg/kg

Examinations

Clinical signs	No
Tissue	bone marrow
Number of animals:	All animals
Number of cells:	250 cells per dose level
Time points:	6, 24, 48 h
Type of cells	erythrocytes in bone marrow
Parameters:	Mitotic index

Results

Neither the variety nor the number of chromosomal aberrations differed significantly from the negative controls; hence citric acid can be considered as non-mutagenic as measured by the cytogenetic test.

Conclusion

The compound produced no detectable significant aberration of the bone marrow metaphase chromosomes of rats when administered orally at the dosage levels employed in this study

Table 17 : Cytogenetic In-Vivo-Test - Chromosomal Analysis in erythrocytes - Acute study

Compound	Dosage (mg/kg)	Time*	No. of animals	No. of cells	Mitotic index % ++	No. of cells w/ breaks **	No. of cells w/ reunion **	No. of cells w/ other aberrations **+	No. of cells w/ aber.**
Intermediate	500	6 24 48	5 5 5	250 236 250	3.67 2.86 5.16	0 0 0	0 1(0.4) 0	0 2pp(0.8) 5pp(2.0)	0 3(1.27) 5(2.00)
High	3500	6 24 48	5 5 5	250 250 250	3.45 3.20 3.90	0 1(0.4) 1(0.4)	0 0 0	0 0 0	0 1(0.4) 1(0.4)
Negative control	Saline	6 24 48	5 5 5	150 150 150	4.47 3.47 5.73	0 0 0	29(19.3)^ 0 0	0 0 0	29(19.33) 0 0
Positive control (TEM)	0.3	24	5	250	1.06	3(1.2)	58(23.2)	>37(14.8) 6f(2.4) 2pp(0.8)	101(40.4)

* Time of kill after dosing (hours)

**Numbers in () are percent aberrations per total cells counted

^ 58% of cells examined in one animal exhibited abnormally large metacentric chromosome and had a diploid number of 41. This was considered to be normal for this animal.

+ Symbols: > = greater than 10 aberations per cell; f=fragments; pp=polyploidy; and pu=pulverisation.

++ Based on a count of at least 500 cells per animal.

Table 18 : Cytogenetic *In Vivo* Test - Chromosomal Analysis in erythrocytes - Subacute study

Compound	Dosage (mg/kg)	No. of animals	No. of cells	Mitotic index % ++	No. of cells w/ breaks **	No. of cells w/ reunion **	No. of cells w/ other aberrations **+	No. of cells w/ aber.**
Intermediate	300	5	250	3.43	0	0	1pp(0.4)	1(0.40)
High	3000	4	200	4.90	0	0	1pp(0.5)	1(0.50)
Negative control	Saline	2	100	4.40	0	0	1pp(1.0)	1(1.00)

** Numbers in () are percent aberrations per total cells counted

++ Based on a count of at least 500 cells per animal.

3.8.2.2 [redacted] (1975)

Study reference:

[redacted]. *(1975), Summary of Mutagenicity Screening Studies: Host Mediated Assay Cytogenetics, Dominant Lethal Assay. Contract FDA 71-268, Compound FDA 71-54 Citric Acid. Litton Bionetics, Inc*

Detailed study summary and results:

In this test, similar to OECD 478, male and female random bred rats from a colony were employed. These animals were 10 - 12 weeks old at the time of use. Ten male rats were assigned to each of 5 groups, 3 dose levels selected as a stated above, a positive control and a negative control.

Administration of the test compound was orally by intubation in both the acute study (1 dose) and in the subacute study (1 dose per 5 days).

Test material	Citric acid
Lot/Batch number	U.S.P Control Number 121M231 IV
Specification	No data
Description	Granular
Purity	No data
Stability	No data
Maximum tolerable dose	
Test Animals	
Species	Rat
Strain	Sprague-Dawley
Source	No data
Sex	Male
Age/weight at study initiation	10 to 12 weeks old at time of use.
Number of animals per group	10 males per dose level including control.
Control animals	Yes

Administration/ Exposure	Oral intubation
Number of applications	1 dose for both acute studies.
	1 dose every 5 days for the subacute study.
Interval between applications	24 h
Post exposure period	8 weeks for acute study and 7 weeks for subacute study.
	Oral
Type	Usually gavage
Concentration	Acute study: 500 mg/kg and 3500 mg/kg
	Subacute study: 300 mg/kg and 3000 mg/kg
Controls	Negative control: Saline
	Positive control: Triethylene Melamine 0.3 mg/kg
Examinations	
Clinical signs	No
Further remarks	Females after termination were subject to necropsy. The uterus was examined for deciduomata (early deaths), late fetal deaths and implantations.

Results

No chromosomal damage occurred in the bone marrow of rats ingesting up to 3 g citric acid/kg bw/day for 5 days

Summary and conclusion

No mutagenic effects seen in the report. No mutagenic potential was detected in a dominant lethal assay in rats in which doses of up to 3 g citric acid/kg bw/day were administered for 5 days. A dominant lethal effect is normally reflected by increased early foetal death when treated males are mated with untreated females. This was not the case in this study.

3.8.3 Human data

No data available.

3.8.4 Other data

No data available.

3.9 Carcinogenicity

3.9.1 Animal data

3.9.1.1 Fukushima S. et al. (1986)

Study reference:

*Fukushima S. et al. (1986), Sodium citrate: A promoter of bladder Carcinogenesis, Japanese journal of cancer research, **77**, pages 1-4*

Detailed study summary and results:

This study was designed to examine the potential promotion of bladder tumorigenesis by sodium citrate. 65 male rats were administered drinking water with (groups 1 and 2) and without (group 3) 0.05 % N-Butyl-N-(4-hydroxybutyl)nitrosamine for 3 weeks and then groups 1 and 3 were fed on a powdered diet containing 5 % sodium citrate for 32 weeks with the group 2 acting as control.

After sacrifice, the bladder was inflated by intraluminal injection of 10 % phosphate-buffered formalin solution. For quantitative analysis, bladder lesions were counted in histological sections stained with hematoxylin and eosin. The total length of basement membrane analysed was measured with a color video image processor and numbers of preneoplastic and neoplastic lesions were expressed per 10 cm of basement membrane.

Test Animals

Species	Rat
Strain	F344
Source	Charles River Japan, inc.
Sex	Male
Age/weight at study initiation	Six weeks old
Number of animals per group	20 / 25 / 20
Control animals	Yes
Administration/ Exposure	Oral
Duration of treatment	32 weeks
Frequency of exposure	Daily
Postexposure period	Not stated

Oral

Type	Dietary
Concentration	5 % of diet
Vehicle	feed
Concentration in vehicle	Not stated
Total volume applied	Not stated
Controls	plain diet

Examinations

Observations	
Clinical signs	No
Mortality	No
Body weight	Yes at study initiation and at study end
Food consumption	No

Water consumption	No
Ophthalmoscopic examination	No
Haematology	No
Clinical Chemisty	No
Urinalysis	Yes All animals Time points: 4,8 and 16 weeks
Sacrifice and pathology	
Organ Weights	yes Organs: Bladder
Gross and histopathology	No
Statistics	Not stated

Results

There were no mortalities in any dose group.

Group 1, fed 5 % sodium citrate, showed reduced average body weights compared to the control group.

According to the report, macroscopically, rats in group 1 had many large bladder tumours and only very small lesions were apparent in group 2 animals, however no further details are provided. No stone formation was observed.

The incidence and number per 10 cm of basement membrane of papilloma and carcinoma were significantly higher in group 1 (papilloma = 95%; 2.06±1.18, respectively; carcinoma = 100%, 1.57±0.50, respectively) than in group 2 (papilloma = 20%; 0.12±0.25, respectively; carcinoma = 8%, 0.05±0.17, respectively). The incidence and number of putative preneoplastic lesions, lesions, papillary or nodular hyperplasia were also significantly increased in group 1 (100%; 7.78±2.47 compared to 44%; 0.46±0.71, respectively in group 2). No lesions were evident in the bladder mucosa of group 3 rats.

Conclusion

In general, it is considered that a high intracellular proliferation and elevation of intracellular concentration of sodium ion is related to cellular proliferation and elevation of intracellular pH increases DNA synthesis of cells *in vitro*. Therefore it is likely that there is a close relation between promotion of bladder carcinogenesis by sodium citrate and the urine changes that are induced.

In the present study, no increase in other electrolytes such as calcium or magnesium ions was observed in the urine of the sodium citrate group.

3.9.1.2 Shibata et al. (1992)

Study reference:

Shibata et al. (1992), The modifying effects of Indomethacin or Ascorbic acid on cell proliferation induced by different types of bladder tumour promoters in rat urinary bladder and fore stomach mucosal epithelium, Japanese Journal of Cancer Research,83:31-39

Detailed study summary and results:

No guideline stated. 65 male rats were split into 13 groups of 5 rats. One group was a negative control. The rats were fed with a powdered basal diet which was supplemented with various test chemicals, apart from the control. After 8 weeks the rats were sacrificed and examined. The examination took the form of microscopic

and macroscopic observation and urinalysis. The weights of the animals and the food and water consumption were measured.

Test material	Ascorbic acid (AsA), Sodium citrate (Na-Cit), Butylated hydroxyanisole (BHA), Indomethacin (IM) and Diphenyl (DP)
Lot/Batch number	Not stated. All were obtained from Wako Pure Chemical Industries Co, Osaka apart from DP which was obtained from Tokyo Kasei Industries, Tokyo.
Specification	Not stated
Description	Not stated
Purity	Not stated
Stability	Not stated
Test Animals	
Species	Rat
Strain	Fischer 344
Source	Charles River Japan, Inc, Atsugi
Sex	Male
Age/weight at study initiation	6 weeks old, about 115g
Number of animals per group	13 groups of 5 rats each
at interim sacrifice	n/a
at terminal sacrifice	All animals sacrificed after 8 weeks.
Control animals	Yes
Administration/ Exposure	Oral
Duration of treatment	8 weeks
Interim sacrifice(s)	n/a
Final sacrifice	All after 8 weeks
Freqency of exposure	5 days/week
Postexposure period	none
	Oral
Type	in food or in drinking water
Concentration	Either 2 % BHA; 2 % BHA+IM (20 ppm in drinking water) ; 2 % BHA + 5 % As A; 5 % Na-AsA ; 5 % Na-AsA+ IM 920 ppm) ; 5 % Na-Cit; 5 % Na-Cit _ 5 % AsA; 0.5 % DP; 0.5 % DP+IM (20 ppm); 0.5 % DP + 5 % AsA; IM (20 ppm) ; 5 % AsA.
Vehicle	Powdered basal diet
Controls	Powdered basal diet with no supplements
Examinations	
Body weight	Yes

Food consumption	Yes	
Water consumption	Yes	
Clinical signs	Yes	
Macroscopic investigations	Palpable masses, skin tumours	
Ophthalmoscopic examination	No	
Haematology	No	
Clinical Chemistry		No
	Number of animals:	All animals, 10 animals/sex/group or other
	Time points:	After 3, 6, 12, 18, 24 months of treatment, end of study or other
	Parameters:	Sodium, potassium, glucose, total cholesterol, urea, blood urea nitrogen, total bilirubin, creatinine, total protein and albumin, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, gamma glutamyl transpeptidase, sorbitol dehydrogenase, methaemoglobin, lipids, hormone (specify hormones), acid/base balance, cholinesterase inhibition.
Urinalysis		Yes
	Number of animals:	All animals
	Time points:	end of study (8 weeks)
	Parameters:	volume, osmolality, pH, Na, K, Ca P, Mg, Crystals
Pathology	No	
Organ Weights		No
	from:	Not applicable
	Organs:	Not applicable
Histopathology	Yes	
	from:	all dose groups
	from:	at terminal sacrifice
	Organs:	Fore stomach, urinary bladder.
Other examinations	DNA synthesis in bladder and forestomach epithelium.	
	Cell proliferation in bladder and fore stomach epithelium.	
Statistics	Student's t test and chi-square test were performed..	

Results

Body weight	Significant weight reduction effects were observed in the body weights of rats fed with BHA, BHA+IM and BHA +ASA, at 2, 4 and 8 weeks, relative to control. Significant weight reduction effects were observed in the body weights of rats fed with DP+IM at 4 and 8 weeks and with DP+AsA at 2, 4 and 8 weeks, relative to control. A significant weight reduction effect was seen in rats fed with AsA at 8 weeks relative to control
Food consumption	No effects
Water consumption	Increased trend in rats receiving BHA, Na-AsA, Na-AsA+IM, Na-Cit, Na-Cit+AsA and DP+AsA
Clinical signs	No effects
Macroscopic investigations	No effects
Ophthalmoscopic examination	n/a
Haematology	n/a
Clinical Chemistry	n/a
Urinalysis	A significant increase or trend towards increase in urine volume was observed in all treated groups, with the exception of the IM and AsA groups. Urinary pH was significantly elevated in rats given Na-AsA or Na-Cit, either alone or in combination with IM or AsA. Treatment with DP+AsA or AsA itself brought about a drop in pH. All groups treated with Na salts demonstrated increase in Na levels and $MgNH_4PO_4$ crystalluria. The urine characteristics in all treated groups were independent of combination with IM or AsA.
Pathology	n/a
Organ Weights	n/a
Histopathology	Hyperplasia was observed in both the bladder epithelium and forestomach epithelium. A Significant number of rats with hyperplasia in bladder epithelium were observed for Na-AsA and Na-Cit+AsA. A significant number of rats with hyperplasia in the forestomach epithelium were observed for BHA and BHA+IM compared to control and for BHA+AsA compared to BHA itself.
Other examinations	DNA synthesis in forestomach epithelium showed a significant effect for BHA, BHA+IM compared to control and for BHA + ASA compared to BHA alone. DNA synthesis in bladder epithelium showed significant effects for BHA, BHA+IM, BHA+AsA, Na-ASa, NA-AsA + IM, Na-Cit, Na-Cit+AsA and DP.
Time to tumours	Not stated

Clinical observation did not reveal any abnormalities or differences between the groups and no deaths occurred.

Significant weight reduction effects were observed in the body weights of rats fed with BHA, BHA+IM and BHA +ASA, at 2,4 and 8 weeks, relative to control.

Significant weight reduction effects were observed in the body weights of rats fed with DP+IM at 4 and 8 weeks and with DP+AsA at 2,4 and 8 weeks, relative to control.

A significant weight reduction effect was seen in rats fed with AsA at 8 weeks relative to control.

Water consumption showed an increased trend in rats receiving BHA, Na-AsA, Na-AsA+IM, Na-Cit, Na-Cit+AsA and DP+AsA

A significant increase or trend towards increase in urine volume was observed in all treated groups, with the exception of the IM and AsA groups. Urinary pH was significantly elevated in rats given Na-AsA or Na-Cit, either alone or in combination with IM or AsA. Treatment with DP+AsA or AsA itself brought about a drop in pH. All groups treated with Na salts demonstrated increase in Na levels and $MgNH_4PO_4$ crystalluria. The urine characteristics in all treated groups were independent of combination with IM or AsA.

Hyperplasia was observed in both the bladder epithelium and forestomach epithelium.

A Significant number of rats with hyperplasia in bladder epithelium were observed for Na-AsA, and Na-Cit+AsA.

A significant number of rats with hyperplasia in the forestomach epithelium were observed for BHA and BHA+IM compared to control and for BHA+AsA compared to BHA itself.

DNA synthesis in forestomach epithelium showed a significant effect for BHA, BHA+IM compared to control and for BHA + ASA compared to BHA alone.

DNA synthesis in bladder epithelium showed significant effects for BHA, BHA+IM, BHA+AsA, Na-ASa, NA-AsA + IM, Na-Cit, Na-Cit+AsA and DP.

AsA was found to enhance the bladder epithelial proliferation associated with Na-Cit treatment and BHA-induced forestomach hyperplasia. AsA caused an increment of DNA synthesis in the bladder epithelium over that with Na-Cit alone. There may be a link between increased hyperplasia and also increased bladder epithelial DNA synthesis due to a combination of Ascorbic acid (AsA) and sodium citrate (Na-Cit).

Conclusion

In conclusion, the present results suggested that IM may exert inhibitory effects on promotion of bladder carcinogenesis and AsA may promote BHA forestomach carcinogenesis. No conclusion was given for Na-Cit with regard to carcinogenesis.

3.9.2 Human data

No data available.

3.9.3 *In vitro* data (e.g. *in vitro* germ cell and somatic cell mutagenicity studies, cell transformation assays, gap junction intercellular communication tests)

No data available.

3.9.4 Other data (e.g. studies on mechanism of action)

No data available.

3.10 Reproductive toxicity

3.10.1 Animal data

3.10.1.1 Bonting S.L., Jansen B.C. (1956)

Study reference:

Bonting S.L., Jansen B.C. (1956), The effect of a prolonged intake of phosphoric acid and citric acid in rats. Voeding 17: 137-148

Detailed study summary and results:

In a metabolism experiment, 24 female rats (age 8 months) who had received basal control diet since weaning were kept on the basal control diet for the first six weeks of the experiment. During the next 8 weeks, 8 animals continued to receive the basal control diet and 8 animals received basal diet plus 1.2 % citric acid (the remaining rats were fed a diet supplemented with a different acid that was being investigated). The experiment was completed with another control period of six weeks during which all animals received the basal control diet.

The reproduction of rats was investigated using animals who received only basal control diet from weaning and using animals who were fed basal diet plus 1.2 % citric acid from weaning.Mating took place when the rats were 32 weeks old and had received the diets for 29 weeks (i.e. from the age of 3 weeks). Eleven weeks later (during which animals had continued to receive basal control diet or base diet supplemented with 1.2 % citric acid) the same animals were mated again. No detrimental effect of the diet containing citric acid on reproduction was found, after either the first or second mating, based on weight of the mothers, the number of living young and stillborn per litter, the average birth weight fo the living young and the number of young left at weaning.

Test Animals

Species	rat
Strain	Albino
Source	No data
Sex	Male/female
Age/weight at study initiation	Not stated
Number of animals per group	In a metabolism experiment, 24 female rats (age 8 months) who had received basal control diet since weaning were kept on the basal control diet for the first six weeks of the experiment. During the next eight weeks, 8 animals continued to receive the basal control diet and 8 animals received basal diet plus 1.2 % citric acid (the remaining rats were fed a diet supplemented with a different acid that was being investigated). The experiment was completed with another control period of six weeks during which all animals received the basal control diet.
Mating	No data
Duration of mating	No data
Deviations from standard protocol	No data
Control animals	yes
Administration/ Exposure	Oral, dietary

Animal assignment to dosage groups	No data
Duration of exposure before mating	29 weeks
Duration of exposure in general P, F1, F2 males, females	90 weeks
	Oral
Type	Dietary supplement
Concentration	Feed containing 1.2 % w/w citric acid.
Vehicle	n/a
Concentration in vehicle	n/a
Total volume applied	n/a
Controls	Basal laboratory diet
Examinations	
Clinical signs	Yes
	General appearance and behaviour
Body weight	Yes
	Average weight of each group: 0, 10 and 20 weeks after weaning
Food/water consumption	Food, weekly. Water, no.
Oestrus cycle	No
Haematology	Blood characteristics: Erythrocytes, leucocytes, lymphocytes, neutrophils, eosinophils, basophiles, young leucocytes and monocytes were counted using blood from 10 animals from the F0 generation (males and females) plus 10 animals from the F1 generation (males and females). At the time of the investigation all animals (F0 and F1) were 32 weeks old and had received basal control diet for 29 weeks. Blood characteristics from animals given basal control diet plus 1.2 % citric acid were not investigated.
Further remarks	Reproduction rates were also measured as part of the study.
	Investigation at 32 weeks old after 29 weeks dosing. After 11 weeks, rats were mated again.
	Observations were weights of mothers, number of living young and number of stillborn per litter, average birth rate weight of living young and number of young left at weaning.

Results

No harmful effects on the growth of two successive generations of rats over a 90-week period. No effects on reproduction, blood characteristics, pathology or calcium was observed although a slight increase in dental attrition was reported

Conclusion

Citric acid has no significant effect on rats at this dose level when dosed over the whole lifetime and has no effect on reproduction.

Metabolism studies showed that chronic dosing of citric acid at 1.2 % of dietary intake has little effect on metabolic processes.

The NOEL for all generations was 470 mg/kg/day.

3.10.1.2 Wright E, Hughes RE (1976b)

Study reference:

Wright E, Hughes RE. (1976b), Some effects of dietary citric acid in small animals, department of biology (University of Wales),Food Cosmet. Toxicol, 14: 561-564

Detailed study summary and results:

Not compliant with a guideline.

Rats received a fully synthetic starch-casein diet which was adjusted to contain the correct amount of calcium and citric acid. Twelve pregnant rats were split into two groups. One group was fed on a diet containing 5 % citric acid the other group was not.

The feeding of the pregnant females continued through pregnancy and the suckling period. The offspring from each group were then fed the same diet as the mother for up to 200 days after birth.

After this period the animals were killed by stunning, followed by decapitation and exsanguination. Blood was collected in heparinised vessels. Organs were dissected out quickly, weighed and treated as was appropriate for the estimation required. Ascorbic acid, total lipids, cholesterol, haemoglobin, inorganic phosphate, plasma iron, serum triglycerides, calcium and alkaline phosphatase were all determined by various analytical means.

Test material	Citric acid, calcium
Lot/Batch number	n/a
Specification	n/a
Description	Solid incorporated into diet
Purity	5 % citric acid and 0.15 % calcium.
Stability	n/a
Test Animals	
Species	rat
Strain	Not stated
Source	Not stated
Sex	Female – pregnant rats. Then groups of male and female offspring
Age/weight at study initiation	Age of pregnant rats not given. Described as young.
Number of animals per group	12 females (of which 6 were controls). The dosed group produced 63 young and the control females produced 64 pups. All pregnant rats were fed a casein-starch diet with the calcium concentration adjusted to 0.15 %. Only one group of 6 pregnant rats received 5 % citric acid in the diet. When the young were born, they received the same diet as their mothers.
Control animals	Yes. 6 control females
Mating period	Not stated

Administration/ Exposure	Oral	
Duration of exposure		
	Rat :	During pregnancy and suckling period. Time not stated.
Post exposure period	The young were fed the same diet as their mother for up to 200 days after birth. The mothers were not fed after giving birth.	
	Oral	
Type	in food	
Concentration	food consumption per day	ad libitum
Vehicle	Dry food	
Concentration in vehicle	All pregnant females received a diet containing 0.15 % calcium. Half of the females received 5 % citric acid in the diet, the other half did not.	
Total volume applied	n/a	
Controls	plain diet	

Results

No maternal deaths were reported.

No effects on the pregnant females were reported

The relative fresh weights of two representative bones (fibula and tibia) was significantly reduced in rats receiving a restricted daily intake of calcium when citric acid was given to mothers during the period of foetal development and for pups, for 200 days following birth

The concentrations of calcium and phosphate in the bone ash of the offspring were not significantly different in the two groups, therefore it appears that elevated dietary citric acid in the presence of minimal calcium intakes, reduces the formation of bone without altering its mineral composition.

A reduction in the packed cell volume and haemoglobin content of the blood was observed in F0 animals. This finding was attributed to the known ability of citric acid to complex with inorganic iron and subsequent reduction in its physiological availability.

No rise in the concentration of citric acid in rat tissue was produced by 5 % dietary citric acid, a finding that lends support to the general supposition that ingested citric acid is rapidly metabolised by the tissues.

Conclusion

Overall, there was no evidence of the induction of deviant metabolism by citric acid. The maximum citric acid intake was approximately 3 g/kg bw/day - about ten times the maximum intake to which man is likely to be exposed.

NOEL = 5 % citric acid (470 mg/kg bw/day)

3.10.1.3 OECD (2001)

Study reference:

OECD (2001), Citric acid: CAS No. 77-92-9 OECD ICCA HPV programme (SIDS dossier) UNEP Publications page 102

Detailed study summary and results:

This report summarises a developmental toxicity study in rats. No guidelines were mentioned in the reporting and no method was detailed. The details which were given are as follows:

Species: rat

Sex: male and females, numbers not stated

Route of administration: not stated, probably oral feed.

Frequency of treatment: daily

Exposure period: days 6 to 15 of gestation

Doses: >241 mg/kg bw/day

GLP: no

Summary and conclusion

No indication of adverse effects on implantation, maternal or foetal survival. The number of abnormalities did not differ from control group. NOEL for maternal toxicity and developmental effects is >241 mg/kg bw/day

3.10.1.4 Wright E, Hughes R.E. (1976a and b)

Study reference:

Wright E., Hughes R.E. (1976a), The influence of a dietary citric acid supplement on the reproduction and survival time of mice and rats. Nutr. Rep. 13: 563-566

Detailed study summary and results:

Not compliant with a guideline.

This report summarises a developmental toxicity study in mice and rats. No guidelines were mentioned in the reporting and no method was detailed. The details which were given are as follows:

Animals were fed 5 % citric acid in the diet previous to, during and subsequent to mating. Number of pups live and still born were noted and the number surviving after 28 days recorded.

Results

There were no effects on number of pups born or their survival up to the point of weaning.

Conclusion

NOEL = 5 % citric acid in diet.

Table 19 : Results of teratology study

	Mice		Rat	
	control	5%	Control	5%
Number of pregnant females	6	6	6	6
Young born	76	78	64	63
Still births	3	4	0	0
No. surviving after 28 days	60	63	54	52

3.10.1.5 ECB (2000)

Study reference:

ECB (2000), IUCLID Dataset Citric Acid

Detailed study summary and results:

No details of the method are available for this study except that it was conducted on female rabbits on days 6-18 of pregnancy. The highest dose group was 425 mg/kg bw/d. The study was not to GLP.

Results and conclusion

There were no indications of teratogenicity or other adverse effects when female rabbits were given up to 425 mg/kg bw/d on days 6 to 18 of pregnancy.

3.10.2 Human data

No data available.

3.10.3 Other data (e.g. studies on mechanism of action)

No data available.

3.11 Specific target organ toxicity – single exposure

3.11.1 Animal data

No data available.

3.11.2 Human data

Please refer to section 3.6.2 RESPIRATORY SENSITISATION human data.

3.11.3 Other data

No data available.

3.12 Specific target organ toxicity – repeated exposure

3.12.1 Animal data

3.12.1.1 Yokotani H. et al. (1971)

Study reference:

Yokotani H. et al. (1971), Acute and Subacute Toxicology Studies of TAKEDA-Citric Acid in Mice and Rats. Journal Takeda Research Laboratory 30 (1) page 25-31.

Detailed study summary and results:

Rats were divided into 4 groups each consisting of 10 animals and maintained on a powdered commercial diet and drinking water, which were given ad libitum. 5 animals were placed in each metal cage and administered the test agent orally by mixing in the diet at rates of 0, 0.2, 2.4 and 4.8 %.

Test material	TAKEDA-citric acid (NCP) and commercially available citric acid
Lot/Batch number	NCP Lot No. M1-100

Specification	Citric acid monohydrate
Description	No data
Purity	NCP 99.8 %
Stability	No data
Test Animals	
Species	Rats
Strain	SD-JCL
Source	No data
Sex	Male
Age/weight at study initiation	Age: 29 – 35 days Weight: 98 – 112 g
Number of animals per group	10 animals/ group (4 groups were used in the study)
Control animals	Yes
Administration/ Exposure	Oral
Duration of treatment	6 weeks
Frequency of exposure	Daily
Postexposure period	24 h
Oral	
Type	In food
Concentration	The test agent was mixed in the diet at rates of 0, 0.2, 2.4 and 4.8 % (0, 1150, 2260, 4670 mg/kg bw).
Vehicle	n/a
Concentration in vehicle	n/a
Total volume applied	n/a
Controls	Plain diet
Examinations	
Observations	
Clinical signs	*Yes* *Behavioural abnormality was observed every day.*
Mortality	Not specified
Body weight	Yes Measured individually once or twice a week.
Food consumption	Yes Measured in a group of 5 animals twice a week.
Water consumption	No
Ophthalmoscopic	No

examination	
Haematology	Yes Number of animals: all animals Time points: end of study Parameters: Haematocrit, haemoglobin concentration, erythrocyte count, total and differential leukocyte count, neutrophile granulocytes.
Clinical Chemisty	Yes Number of animals: all animals Time points: end of study Parameters: glucose, total cholesterol, blood urea nitrogen, total bilirubin, creatinine, total protein, albumin, alkaline phosphatase, globulin, albumin/ globulin ratio, calcium ions, LDH, GOT, GPT, BSP retention.
Urinalysis	Yes Number of animals: all animals Time points: 41st feeding day Parameters: protein
Sacrifice and pathology	
Organ Weights	Yes Organs: liver, kidneys, adrenals, testes, thyroid, spleen, brain, heart lungs, prostate, thymus, hypophysis
Gross and histopathology	Yes All dose groups Organs: liver, kidneys, adrenals, testes, thyroid, spleen, brain, heart lungs, prostate, thymus, hypophysis.

Results

Observations	
Clinical signs	No behavioural abnormalities throughout the feeding period.
Mortality	No mortalities at any dose
Body weight gain	The bodyweight gain in animals fed 1.2, 2.4 and 4.8 % NPC diet was found to be depressed slightly from the end of 1 or 2 weeks and from the beginning of 1 week respectively. The % body weight gain in animals with the respective feeding was 98.8, 92.6 and 94 % of that of the control group.
Food consumption and compound intake	The daily food intake/100 g bw in animals fed 1.2, 2.4 and 4.8 % NPC diet was depressed by 0.7, 2.6 and 4 % respectively of the levels in the control animals. The mean daily NPC intake measured in the respective groups were 1.15, 2.26 and 4.67 g/kg/day.
Ophtalmoscopic examination	n/a
Blood analysis	
Haematology	Slight but not significant decrease in the counts of erythrocytes and leucocytes, haematocrit value and haemoglobin content were detected

	in the animal groups given higher doses. Analysis of the haemogram revealed only relative decrease in lymphocytes and the relative increase in neutrophile granulocytes.
Clinical chemistry	The total plasma protein tended to decrease in the treated animals and the same decrease in the 2.4 % groups was statistically significant. The decrease in the plasma albumin and the ratio of albumin/globulin in the plasma were found only in animals treated with the 4.8 % NPC diet. The highest dose group exhibited some decrease in the plasma cholesterol level and elevated activity of GOT in the serum. Some of the animals given higher doses showed a slightly prolonged BSP retention. The blood level of calcium ion in the treated animals showed some decrease which proved not to be either significant or dose dependent.
Urinalysis	One of the animals fed the 4.8 % NPC diet showed an increase presence of protein in the urine and the occult blood reaction was demonstrated in 4 of 5 animals fed the 4.8 % NPC diet. Many lymphocyte-like cells were detected in the sediment of the urine with the increased presence of protein.
Sacrifice and pathology	
Organ weights	Abnormality in the size of the testis was found in one animal fed the 1.2 % NPC diet and one animal fed the 4.8 % NPC diet. The thymus and the spleen of animals fed the 4.8 % NPC diet showed some decline in weight and the decrease in thymus weight was significant. The prostate in animals fed the 1.2 % NPC diet and the thyroid gland in animals fed the 4.8 % NPC diet showed some decrease in weight.
Gross and histopathology	There were yellowish-white miliary tubercles on the inner surface of the right hepatic lobe. Slight atrophy of the cortex of the thymus and of the lymph follicle of the spleen in animals fed the 4.8 % NPC diet was observed histologically. However, other histopathological lesions especially those reflecting a decrease in organ weight were found in the organs of any of the rats fed the 1.2, 2.4 and 4.8 % NPC diet.

Summary and Conclusion

Daily oral administration of NPC produced no behavioural abnormalities except a slight depression of body weight gain and daily food intake. Morphological and biochemical examinations of the blood and urine demonstrated some mild but abnormal findings. However many of the findings could not be ascribed to the toxic effect of NPC. Though the blood levels of calcium ion in the treated animals decreased slightly, the decrease was not dose dependent. The urine is normally calcium free but calcium rapidly appears in the urine when the plasma concentration is elevated by even a slight degree. The presence of occult blood in the urine of rats given the highest dose of NPC suggests an increased output of calcium salts. However, no evidence to support the suggestion was presented in the present experiment. No deleterious effects of NPC on organs were observed histologically under the present experimental conditions.

The effects observed were not thought to be caused by the test material.

NOAEL: 2260 mg/kg bw/day

Table 20: Results of repeated dose toxicity study

Parameter	Control	1.15 g/kg/day	2.26 g/kg/day	4.67 g/kg/day	dose-response +/-
Sex	m	m	m	m	
Number of animals examined	5	5	5	5	
Mortality	0	0	0	0	
Clinical signs					
Body weight		↓ (end of 1 or 2 weeks)	↓ (end of 1 or 2 weeks)	↓ (beginning of week 1)	-
Food consumption		↓	↓	↓	-
Clinical chemistry* - plasma protein			↓		-
Clinical chemistry – plasma albumin, albumin/ globulin ratio				↓	-
Haematology* - erythrocytes, leucocytes, haemtocrit, haemoglobin				↓	-
Urinalysis*- protein				↑	-
<u>Organ- testes</u> organ weight*		↑		↑	-
<u>Organ- thymus</u> organ weight*				↓	-
Gross pathology*				Slight atrophy (number of animals not reported)	-
<u>Organ- spleen</u> organ weight*				↓	-
Gross pathology*				Slight atrophy (number of animals not reported)	-
<u>Organ- prostate</u> organ weight*		↓			-
<u>Organ- thyroid gland</u> organ weight*				↓	-

↑or ↓for increased or decreased.

3.12.1.2 Wright E., Hughes R.E. (1976b)

Study reference:

Wright E., Hughes R.E. (1976), Some effects of dietary citric acid in small animals, department of biology (University of Wales),Food Cosmet. Toxicol, 14: 561-564.

Detailed study summary and results:

Not compliant with a guideline.

First part of study: Young rats, initial weight about 240g

Second part of study : Slightly older rats, initial weight about 340g

The first group were treated with 4 % dietary citric acid and given a calcium-restricted (0.2 %) diet.

The second group were treated with 4 % citric acid, maintained in a state of calcium-sufficiency.

Rats received a fully synthetic starch-casein diet which was adjusted to contain the correct amount of calcium and citric acid.

After this period the animals were killed by stunning, followed by decapitation and exsanguination. Blood was collected in heparinised vessels. Organs were dissected out quickly, weighed and treated as was appropriate for the estimation required. Ascorbic acid, total lipids, cholesterol, haemoglobin, inorganic phosphate, plasma iron, serum triglycerides, calcium and alkaline phosphatase were all determined by various analytical means.

Test material	Citric acid, calcium
Lot/Batch number	Not stated
Specification	Not stated
Description	solid
Purity	5 % of citric acid in diet, 0.15 % calcium in diet
Stability	Not stated
Test Animals	Non-entry field
Species	Rat
Strain	Not stated
Source	Not stated
Sex	Males
Age/weight at study initiation	First part of study: Young rats, initial weight about 240 g Second part of study : Slightly older rats, initial weight about 340 g
Number of animals per group	12 rats in first part , 10 rats in second part of study. The first group were treated with 4 % dietary citric acid and given a calcium-restricted (0.2 %) diet. The second group were treated with 4 % citric acid, maintained in a state of calcium-sufficiency.
Control animals	Yes
Administration/ Exposure	Oral
Duration of treatment	60 days
Frequency of exposure	Not stated
Postexposure period	Not stated
Oral	
Type	in food
Concentration	food consumption per day, ad libitum
Vehicle	Given as dry food
Concentration in vehicle	n/a
Total volume applied	n/a

Controls	plain diet
Examinations	
Observations	In the rats receiving the low-calcium diet (0.2 %) and 4 % citric acid there was a significant depression of the growth rate. There was no effect on relative organ weights. Serum alkaline phosphatase (often regarded as an index of tissue damage) was unaltered by citric acid.
Clinical signs	yes
Mortality	yes
Body weight	yes (60 days). Statistically significant (student T-test)lower weight difference in animals fed with 4 % citric acid and 0.2 % calcium
Food consumption	no
Water consumption	no
Ophthalmoscopic examination	no
Haematology	yes number of animals: all animals time points: end of study Parameters: packed cell volume, statistically significant lower level in animals fed with 4 % citric acid and 0.2 % calcium
Clinical Chemisty	yes number of animals: all animals time points: end of study Parameters: total cholesterol, lipids, ascorbic acid, inorganic phosphate, plasma iron, serum triglycerides, calcium and alkaline phosphatase
Urinalysis	no
Sacrifice and pathology	
Organ Weights	yes organs: liver, kidneys, adrenals, spleen.
Gross and histopathology	no
Other examinations	Fibula and tibia fresh weights and calcium in the ash after incineration
Statistics	Student T-test used.
Results	
Observations	
Clinical signs	
Mortality	no mortalities at any dose
Body weight gain	In the group fed with 4 % citric acid and low calcium (0.2 %), the weight of the test animals was significantly lower than the controls

Food consumption and compound intake	Not stated
Ophtalmoscopic examination	n/a
Blood analysis	
Haematology	Statistically significant lower level in animals fed with 4 % citric acid and 0.2 % calcium
Clinical chemistry	Serum alkaline phosphatase (often regarded as an index of tissue damage) was unaltered by citric acid.
Urinalysis	n/a
Sacrifice and pathology	
Organ weights	No effects
Gross and histopathology	Not stated
Other	Significant depression in growth rate in rats fed with 4 % citric acid and low calcium in diet.

Summary and conclusion

Statistically significant differences were observed in; final body weight and packed cell volume of test animals fed 4 % citric acid and low calcium. No statistically significant differences were observed in the test group being fed with 0.7 % calcium and 4 % citric acid.

The packed cell volume was not really explained with absolute certainty. The final weight difference was due to depression of growth rate. The bone weights did not seem to be affected relative to the control so the weight difference can't really be assigned to lighter bones.

These results don't appear to indicate any serious toxic effects for citric acid. In fact, the maximum citric acid intake was approximately 3 g/kg bw/day - about ten times the maximum intake to which man is likely to be exposed.

NOAEL : 2000 mg/kg bw/day

Table 21 – 1: Results of clinical chemistry haematology

Parameter changed	Unit	Low dose ontrols	Low dose (0.2 % calcium), 4 % citric acid	High dose control	High dose (0.2 % calcium), 4 % citric acid
29 weeks after start of treatment					
Males					
Packed cell volume	mg/100ml	43.8 ± 1.5	*40.0 ± 1.2 (↓)	48.4 ± 1.3	48.2 ± 1.5

* $p < 0,05$

Table 22: Results of repeated dose toxicity study

Parameter	Low dose control	Low dose (0.2% calcium), 4% citric acid	High dose control *(no statistical differences between control and test animals were observed)*	High dose (0.7% calcium), 4 % citric acid
Sex	m	m	m	m
Number of animals examined	12	12	12	12
Mortality	0	0	0	0
clinical signs	Not stated	Not stated	Not stated	Not stated
body weight				
Initial	233 ± 8 g	242 ± 7 g	345 ± 7 g	352 ± 9 g
Final	365 ± 7 g	**337 ± 5 g (↓)	466 ± 10 g	458 ± 10 g
food consumption	Not stated	Not stated	Not stated	Not stated
clinical chemistry	No statistically significant differences	No statistically significant differences	No statistically significant differences	No statistically significant differences
haematology	43.8 ± 1.5	*40.0 ± 1.2 (↓)	48.4 ± 1.3	48.2 ± 1.5
urinalysis*	Not stated	Not stated	Not stated	Not stated
organ weight*	No statistically significant differences	No statistically significant differences	No statistically significant differences	No statistically significant differences
Fibula and tibia				
Relative fresh weight (% body weight)	0.155 ± 0.005	0.154 ± 0.004	0.121 ± 0.002	0.122 ± 0.003
Calcium in ash (%)	33.7 ± 0.5	33.2 ± 0.6	31.3 ± 0.59	30.7 ± 0.89
gross pathology*	Not stated	No significant Not stated	Not stated	Not stated
microscopic pathology*	Not stated	Not stated	Not stated	Not stated

* $p < 0.05$

** $p < 0.02$

3.12.1.3 Krop S., Gold H. (1945)

Study reference:

Krop S., Gold H. (1945), On the toxicity of hydroxyacetic acid after prolonged administration: Comparison with its sodium salt and citric and tartaric acids. J Am Pharm. Assoc. 34: 86-89.

Detailed study summary and results:

3 dogs were dosed with 1380 mg citric acid daily, in a gelatin capsule directly after feeding with 100 g meat, for a period of 112 to 120 days. No control was used. Certain blood parameters, urinalysis and some histopathology examinations were performed:

Examinations

Observations	
Clinical signs	*Yes*
	General appearance and behaviour
Mortality	*Yes*
Body weight	*Yes*
	weekly
Food consumption	*No*
Water consumption	*No*
Ophthalmoscopic examination	*no*
Haematology	yes All 3 animals weekly
	Parameters: blood NPN and creatinine
Clinical Chemisty	yes all animals
	Every 3 weeks
	Parameters: serum carbon dioxide, power and kidney function
Urinalysis	yes

Sacrifice and pathology

Organ Weights	no
Gross and histopathology	One dog,
	Liver, kidney and lung examined
Other examinations	*None*
Statistics	No data

Results

No effects were noted in any of the parameters measured.

Citric acid is not harmful to dogs at the dose level specified.

Deficiencies:

Weight of dogs at start of study not specified, therefore a specific dose level cannot be ascertained for the NOEL.

One dog had been previously dosed with hydroxyacetic acid in another study discussed in the paper.

Table 23: Results of repeated dose toxicity study

Parameter	1380 mg	dose-response +/-
number of animals examined	3	-
No of doses	103, 108, 109	-
Period of administration	112, 119, 120 days	-
Mortality	0	-
NPN mg %	0	-

3.12.1.4 Wright E., Hughes R.E. (1976a)

Study reference:

Wright E., Hughes R.E. (1976a), The influence of a dietary citric acid supplement on the reproduction and survival time of mice and rats. Nutr. Rep. 13: 563-566.

Detailed study summary and results:

Male albino mice (11 per dose group) were fed a 5 % dietary supplement of citric acid (7500 mg/kg bw/d) to determine if the substance had an effect on survival time.

Mice were dose until total mortality occurred.

Sexually immature and sexually mature mice were studied.

Control mice were fed diet without the supplement of citric acid. Body weights were measured at the 14 month observation.

Results

Observations

Clinical signs	*None stated*
Mortality	*Mean survival time*
	Sexually immature animals:
	Control: 16.73 ± 1.85 months
	*Dosed: *12.73 ± 2.00 months*
	Sexually mature animals:
	Control: 16.46 ± 1.24 months
	*Dosed: *10.80 ± 1.26 months*
	** difference between means $P < 0.05$*

***difference between means P < 0.01*

Body weight gain

Sexually immature animals:

Control (initial body weight): 22.6 ± 0.3 g

Control (body weight 14 months): 44.3 ± 0.8 g

Dosed (initial body weight): 21.7 ± 0.6 g

*Dosed (body weight 14 months): *39.9 ± 1.5 g*

Sexually mature animals:

Control (initial body weight): 39.0 ± 0.9 g

Control (body weight 14 months): 39.1 ± 0.6 g

Dosed (initial body weight): 37.6 ± 0.8 g

*Dosed (body weight 14 months): **31.8 ± 1.9 g*

** difference between means P < 0.05*

***difference between means P < 0.01*

Food consumption and compound intake

No significant difference in food consumption between groups

Summary and Conclusion

The results appear to indicate that citric acid at a level of 5 % in the diet for a prolonged period depressed bodyweight gain and reduced survival time

Effects are more marked in animals that had attained sexual maturity before exposure.

It is unlikely that the effects are a direct influence of citric acid on cellular metabolism.

Unpublished studies by the author has shown that at this level of uptake, citric acid has no effect on typical "toxicity" indices such as plasma enzymen levels, organ weights etc.

The effects observed may be due to the substance having an effect on physiological availability of another cation such as calcium.

LOAEL: 7500 mg/kg bw/day

Table 24 : Body weight results

	Initial bodyweight (g)	Bodyweight at 14 months (g)
Sexually immature		
control	22.6 ± 0.3	44.3 ± 0.8
dosed	21.7 ± 0.6	39.9 ± 1.5
Sexually mature		
control	39.0 ± 0.9	39.1 ± 0.6
dosed	37.6 ± 0.8	31.8 ± 1.9

3.12.1.5 Packman E.W. et al. (1963)

Study reference:

Packman E.W. et al. (1963), Comparative sub-acute toxicity for rabbits of citric, fumaric and tartaric acids. Toxicology and Applied Pharmacology 5:163-167.

Detailed study summary and results:

30 New Zealand rabbits were fed either standard laboratory diet or diet containing the equivalent of 5.0 % citric acid (15 animals per dose group) for a period of 150 days.

Test Animals	Non-entry field
Species	*rabbit*
Strain	*New Zealand*
Source	*Not specified*
Sex	*male*
Age/weight at study initiation	*1 -3 kg*
Number of animals per group	*15 animals per group (control and 1 dose group only)*
Control animals	Yes
Administration/ Exposure	Oral, dietary
Duration of treatment	150 days
Frequency of exposure	daily
Postexposure period	None
Oral	
Type	Dietary supplement
Concentration	7.7 % sodium salt, equivalent to 5.0 % citric acid
	Approximately equivalent to 2825 mg/kg bw/day
Vehicle	None
Controls	Rockland rabbit diet only
Examinations	
Observations	
Clinical signs	*Yes*
	General appearance and behaviour
Mortality	*Yes,*
Body weight	*Yes*
	weekly
Food consumption	*Yes*
	weekly
Water consumption	*No*

Ophthalmoscopic examination	*no*
Haematology	Yes, 60 days after initiation of study
	Erythrocyte, leucocyte and differential leucocyte counts, blood sugar and non-protein nitrogen determinations on 5 rabbits
Clinical Chemistry	No
Urinalysis	Yes, 60 days after initiation of study
	Colour, appearence, specific gravity, sugar, albumin, alkalinity and microscopic constituents on 5 rabbits.
Sacrifice and pathology	
Organ Weights	Yes, on animals taken for gross pathology
	Adrenals, bladder, brain, heart, kidneys, liver, lung, prostate, spleen, stomach, testes and thyroid
Gross and histopathology	On 2 animals after 30 days and 1 after 60 days.
	Histopathology on testes
	Half of remaining animals at 100 days.
	Histopathology on testes, liver and kidney
	All remaining animals sacrificed at end of study
	Gross and histologic studies performed.

Results

Observations	
Mortality	*There were 3 deaths in the control group and 2 in the dose group during the course of the study. There was no apparent connection between feeding of the test substance and mortality.*
Clinical signs	*No effects*
Body weight	*No significant difference from control group*
Ophtalmoscopic examination	*Not specified*
Haematology	After 60 days, all counts were within normal limits. Blood sugar and nonprotein nitrogen values were normal.
Urinalysis	No significant difference between control and dose group.
Sacrifice and pathology	
Organ weights	*No significant difference in weights between control and dose group.*
Gross and histopathology	*No significant differences between control and dose group.*

Summary and conclusion

No significant effects were noted from dosing at 5 % citric acid over a period of 150 days.

NOEL = 5% citric acid in diet (approximately equivalent to 2825 mg/kg bw/day)

Table 25 : Results of repeated dose toxicity study

Parameter	Control	2825 mg/kg/day	dose-response +/-
Deaths (Total)	3	2	None
body weight (week 22)	3.07 kg	2.95 kg	None
Mean total food consumption	17.2 kg	17.8 kg	None
Organ weight (g/kg):			
Testes	1.30	1.59	None
Thyroid	0.14	0.1	None

3.12.1.6 Horn H.J. et al. (1957)

Study reference:

Horn H.J. et al. (1957), Safety of adipic acid as compared with citric and tartaric acid. J Agric. Fd. Chem. 5(10):759.

Detailed study summary and results:

20 male albino rats were dosed at 1280 or 1960 mg/kg bw/day in diet for 104 weeks. 20 male and 10 female rats were fed the basal laboratory diet only as a control.

Rats were observed for clinical signs of toxicity, food consumption, body weight gain, mortalities and gross histopatholgy including organ weight.

Test Animals

Species	*rat*
Strain	*albino*
Source	*Carworth Farm*
Sex	*Male/female*
Age/weight at study initiation	*Male: 60 g* *Female: 50 g*
Number of animals per group	*Control: 20 males, 10 females* *Dose groups 20 males only*
Control animals	Yes
Administration/ Exposure	Oral, dietary
Duration of treatment	2 years
Frequency of exposure	daily
Postexposure period	None

<u>Oral</u>

Type	Dietary supplement

Concentration	3 % or 5 % citric acid
Vehicle	None
Total volume applied	*3 %: 512 mg/rat/day which approximates to 1280 mg/kg bw/day*
	5 %: 784 mg/rat/day which approximates to 1960 mg/kg bw/day
Controls	Basal laboratory diet
Examinations	
Observations	
Clinical signs	*Yes*
	General appearance and behaviour
Mortality	*Yes, gross autopsy on all mortalities during the study*
Body weight	*Yes*
	weekly
Food consumption	*Yes*
	weekly
Water consumption	*No*
Ophthalmoscopic examination	*no*
Haematology	No
Clinical Chemisty	No
Urinalysis	No
Sacrifice and pathology	
Organ Weights	Yes, half of dose group
	Brain, thyroid, lungs, heart, liver, spleen, kidneys, adrenals, stomach, and testes
Gross and histopathology	On representative number of animals in each group.
	Thyroid, lungs, heart, liver, spleen, kidneys, adrenals, stomach, small intestine, large intestine, pancreas, bone marrow, testes or ovaries and uterus
Results	
Observations	
Clinical signs	*Throughout the study, and especially in the last 6 months all groups including controls showed the following symptom: wheezing, blood-tinged crust about the nose and eyes, body sores.*
Mortality	*6 animals dosed at 1280 mg/kg bw/day and 4 animals dosed at 1960 mg/kg bw/day died during the study. The number of deaths were not significant in comparison with the controls.*
Body weight gain	*During the rapid growth period, weight gains in the 3 and 5 % citric acid groups were significantly less than controls (percentage difference not stated).*
Food consumption and	*There was a slight but consistent reduction in food consumption in the*

compound intake	*group dosed with 1960 mg/kg bw/day.*
Sacrifice and pathology	
Organ weights	*No difference in organ weights between dosed animals and controls.*
Gross and histopathology	*No significant gross pathology observed that could be related to clinical symptoms observed. All other examinations were within normal limits for male rats.*

Summary and conclusion

Rats dosed with citric acid showed a significant reduction in body weight gain in comparison with controls. This was probably due to a similar reduction in food consumption during the course of the study. No other significant effects were noted.

As the adverse effect is based on reduction in bodyweight gain from reduced food consumption,which may be due to adverse taste in diet the NOAEL is 1960 mg/kg bw/day.

Table 26 : Results of repeated dose toxicity study

Parameter	Control		1280 mg/kg/day	1960 mg/kg/day	dose-response +/-
Sex	m	f	m	m	m
Survival (%)	82.5	98.9	92.6	95.0	-
Deaths (Total)	12		6	4	-
Body weight (week 0) (g)	59	49	62	61	-
body weight (week 104) (g)	440	321	417	397	+
food consumption (g/rat/day)	16.8	14.2	17.1	15.7	-

3.12.1.7 Bonting S.L., Jansen B.C. (1956)

Study reference:

Bonting S.L., Jansen B.C. (1956), The effect of a prolonged intake of phosphoric acid and citric acid in rats. Voeding 17: 137-148.

Detailed study summary and results:

Male and female albino rats were fed a diet containing 1.2 % citric acid. The rats were dosed over a period covering lifetime and 3 successive generations. The effects on several parameters were studied including reproduction and metabolism.

Test Animals

Species	rat
Strain	albino
Source	Not stated
Sex	Male/female
Age/weight at study	Not stated

initiation	
Number of animals per group	Rat metabolism studies: In a metabolism experiment, 24 female rats (age 8 months) who had received basal control diet since weaning were kept on the basal control diet for the first six weeks of the experiment. During the next eight weeks, 8 animals continued to receive the basal control diet and 8 animals received basal diet plus 1.2 % citric acid (the remaining rats were fed a diet supplemented with a different acid that was being investigated). The experiment was completed with another control period of six weeks during which all animals received the basal control diet.
Control animals	Yes
Administration/ Exposure	Oral, dietary
Duration of treatment	Rat lifetime including reproduction and successive generations (3 generations studied)
Frequency of exposure	daily
Postexposure period	None
Oral	
Type	Dietary supplement
Concentration	1.20 % citric acid
Vehicle	None
Controls	Basal laboratory diet
Examinations	
Observations	
Clinical signs	Yes General appearance and behaviour
Mortality	Yes, gross autopsy on all mortalities during the study
Body weight	Yes Average weight of each group: 0, 10 and 20 weeks after weaning
Food consumption	Yes weekly
Water consumption	No
Ophthalmoscopic examination	no
Haematology	Yes Blood characteristics: Erythrocytes, leucocytes, lymphocytes, neutrophils, eosinophils, basophiles, young leucocytes and monocytes were counted using blood from 10 animals from the F0 generation (males and females) plus 10 animals from the F1 generation (males and

	females). At the time of the investigation all animals (F0 and F1) were 32 weeks old and had received basal control diet for 29 weeks. Blood characteristics from animals given basal control diet plus 1.2 % citric acid were not investigated.
	After the study total blood was analysed for inorganic phosphorus, calcium, total base, carbon dioxide in blood serum, chloride and alkaline phosphatase activity in serum
Clinical Chemisty	No
Urinalysis	Yes during metabolism experiments.
	Over a period of 7 days
	pH, ammonia, urea, total N and inorganic phosphate, calcium, total base and citric acid content.
	Faeces was also analysed for total faecal phosphorus, calcium and total N
Sacrifice and pathology	
Organ Weights	Not stated
Gross and histopathology	Gross anatomical changes
	Histological examination of liver, kidney, spleen, adrenals, testicles, skeletal muscle and femur
Other examinations	Total phosphorus, sodium and potassium, were analysed in tissues. Alkaline phosphatase activity of the kidney. Phosphorus and calcium in the tibiae.
	Dental erosion.
Statistics	Bilateral tail probability, i.e. probability that the difference is accidental was calculated in each instance. Difference considered to be significant if $p < 0.05$.
Further remarks	Reproduction rates were also measured as part of the study.
	Investigation at 32 weeks old after 29 weeks dosing. After 11 weeks rats were mated again.
	Observations were weights of mothers, number of living young and number of stillborn per litter, average birth rate weight of living young and number of young left at weaning.
	Rat metabolism studies:
	24 females (age 8 months) from the control group fed on diet. After 6 weeks, 8 animals from this group were kept on the laboratory diet and 8 animals were fed diet containing 1.2 % citric acid for a further 6-week period.
	The metabolisms studies determined calcium, phosphorus and nitrogen balances, by comparison of intake against elimination in urine and faeces (mg/day).

Results

Observations

Clinical signs	No effects stated
Mortality	No effects stated
Body weight gain	No effect on growth in three successive generations of rat observed.
Food consumption and compound intake	No effect stated
Ophtalmoscopic examination	Not specified
Blood analysis	
Haematology	No significant differences noted.
Clinical chemistry	Not specified
Urinalysis	Analysis of urine was only carried out during the metabolism experiments: Citric acid did not exert an influence on phosphorus balance or excretion. It did not cause a significant change in either calcium balance or excretion. There were no effects on ammonia or urea excretion. There was no effect on urinary fixed base excretion. There was no effect on either acidity or pH of urine. There was no significant increase in the amount of citric acid excreted in urine from the dosed animals to that of the control group.
Sacrifice and pathology	
Organ weights	No difference in organ weights between dosed animals and controls.
Gross and histopathology	No significant gross pathology observed that could be related to clinical symptoms observed. All other examinations were within normal limits for male rats.
Other	Reproduction studies: No detrimental effects on either mothers or young after 2 successive matings. Tissue analysis: Performed in 2 series; Series 1consisted of twelve 15-month old female rats, series 2 consisted of seven 6-month old male rats from the dosed group. No effects were observed in inorganic phosphorus, calcium, total base, chloride and alkaline phosphatase activity of the kidney, in fresh weight, total P and K of the liver, in K content of the muscle and in P content of tibia. The Na content of the liver was decreased and the total P content of the muscle was decreased in both male and female rats. This was countered by an increase in Na content of muscle.

Summary and conclusion

Citric acid has no significant effect on rats at this dose level when dosed over the whole lifetime and has no effect on reproduction.

Metabolism studies showed that chronic dosing of citric acid at 1.2 % of dietary intake has little effect on metabolic processes.

Based on rats and dietary intake specified in Packman E.W. et al. (1963), it is estimated that a dose of 1.2 % in diet would be approximately equivalent to 188 mg/rat/day or 470 mg/kg/day.

NOEL : 470 mg/kg bw/day

Table 27 : Results of repeated dose toxicity study

Parameter	Control		1280 mg/kg/day	1960 mg/kg/day	dose-response +/-
	m	f	m	m	m
Mortality	82.5	98.9	92.6	95.0	-
Deaths (Total)	12		6	4	-
body weight (week 104)	440	321	417	397	+
food consumption (g/rat/day)	16.8	14.2	17.1	15.7	-

3.12.2 Human data

No data available.

3.12.3 Other data

No data available.

3.13 Aspiration hazard

No data available.

3.13.1 Human data

No data available.

3.13.2 Other data

No data available.

4 ENVIRONMENTAL HAZARDS

4.1 Degradation

4.1.1 Ready biodegradability (screening studies)

4.1.1.1 Gerike P, Fischer WK (1979)

Study reference:

Gerike W K, 1979 A correlation study of biodegradability determinations with various chemicals in various tests. , Ecotoxicol. And Environ. Safety, V3: 159-173

Detailed study summary and results:

Table 28.

Guideline/ Test method	Test type	Model for	Inoculation	Conc. (mgC/l)	Degradation Duration (d)	Degree (%)	Parameter
OECD 302 A 88/302/EEC Part C.10	OECD coupled unit test	Sludge from communal STP	Effluent or 2.5 g sludge/l	12	Variable	93	DOC removal
OECD 302 B 88/302/EEC Part C.9	Zahn-Wellens	Industrial STP	1 g sludge/l	400	14	85	DOC removal
OECD 301 A 84/449/EEC Part C.4	AFNOR T90-302	Polluted river	$(5 \pm 3) \times 10^5$ germs/ml	40	42	100	DOC removal
OECD 301 B 84/449/EEC Part C.5	Modified sturm test	Surface water	Effluent after acclimatation	10	28 (42)	97	CO_2 evolution
						100	DOC removal
OECD 301 D 84/449/EEC Part C.6	Closed bottle test	Surface water	1 drop of effluent/l	2	30	90	BOD
OECD 301 E 84/449/EEC Part C.3	Modified OECD Screening test	Surface water	0.05% effluent	3 - 20	19	100	DOC

DOC: dissolved organic carbon, COD: chemical oxygen demand, BOD: biochemical oxygen demand

The biological degradability (ready biodegradability and inherent biodegradability) of citric acid in the aquatic environment was studied by seven different methods including, Zahn-Wellens, Sturm test, Modified OECD screening test, Closed bottle test and Coupled units OECD Confirmatory test. This study was conducted before the requirement for GLP and was investigated by Gerike and Fischer in 1979 at the Department of Ecology, Henkel kGaA, Düsseldorf, West Germany. The parameters to determine the biodegradability were the biochemical oxygen demand (BOD), the carbon dioxide production (CO2), the chemical oxygen demand (COD), or the dissolved organic carbon (DOC). Tests were conducted over a period of minimum 14 days and the nature of the inoculum were domestic sewage sludge, effluent from industrial sewage treatment plant and surface water.

Conclusion

The study of Gerike and Fischer was designed to compare the different biodegradability tests. The results showed that all the tests are in good agreement, showing high percentage of biodegradability (>85%). The active substance is classified as inherently and readily biodegradable.

Ready biodegradability:

AFNOR T90 – 302 test (OECD 301A)

In this test modelled for polluted river, concentrations are rigidly fixed [(5 ± 3) × 105 germs/ml)]. The results are stated as percentage DOC removal after 42 days. This test gave a citric acid degradation rate of 100%.

Modified Sturm test (OECD 301B)

Besides the conventional carbon dioxide production (CO2), the dissolved organic carbon (DOC) removal was followed as a further biodegradation measure. It is the only one test to employ a preacclimation procedure, therefore two tests duration are given, 28 days without and 42 days including the acclimation period of 14 days. The preacclimation was modified in such a way that 20 mg/l of material, 20 mg/l of yeast extract, and 10% of sewage treatment plant effluent rather than raw sewage were added to BOD water in order to avoid anaerobic conditions. This test gave a citric acid degradation rate of 97% based on CO2 evolution and 100% degradation based on DOC removal.

Closed bottle test (OECD 301D)

The results are reported as the biochemical oxygen demand as a percentage of the theoretically possible amount (theoretical biochemical oxygen demand, BODT) after 30 days at the standard test concentration of 2 mg/l. The resulting degradation of citric acid was determined at 90%.

Modified OECD Screening Test (OECD 301E)

This test was designed for surface water. The results are reported as percentage DOC removal after 19 days. This test reported a citric acid value for degradation of 100%.

4.1.2 BOD_5/COD

4.1.2.1 Gerike P, Fischer WK (1979)

Study reference:

Gerike P, Fischer W K, 1979 A correlation study of biodegradability determinations with various chemicals in various tests. , Ecotoxicol. And Environ. Safety, V3: 159-173

Detailed study summary and results:

Please refer to the summary in 4.1.1.

4.1.3 Aquatic simulation tests

No data available.

4.1.4 Other degradability studies

INHERENT AND ENHANCED BIODEGRADABILITY

4.1.4.1 Gerike P, Fischer WK (1979)

Study reference:

Gerike P, Fischer W K, 1979 A correlation study of biodegradability determinations with various chemicals in various tests. , Ecotoxicol. And Environ. Safety, V3: 159-173

Detailed study summary and results:

Please refer to the summary in 4.1.1.

OECD coupled units test (OECD 302A)

It is the best model of all the tests given here for a communal sewage treatment plant, and it is the only one to work under steady-state conditions. Test was realised with a full load (2.5 g/l of dry mater) of sludge from a communal sewage treatment plant and mean dissolved organic carbon (DOC) removal was reported. A value of 93% degradation of citric acid was obtained with this test.

Zahn – Wellens test (OECD 302B)

This model was designed to evaluate the removability of industrial chemicals released by point discharge through industrial sewage treatment plants into the environment. The percentage of dissolved organic carbon (DOC) removal is measured after 14 days period. This test indicated 85% elimination after 1 days and 98% elimination after 48 hours respectively, suggesting rapid absorption and removal of the active substance by sewage sludge.

HYDROLYSIS

4.1.4.2 O'Connor B and Mullee D (2006a)

Study reference:

O'Connor B and Mullee D, 2006 Determination of General Physico-Chemical Properties and Spectra, Safepharm Laboratories, 2189/0001.

Detailed study summary and results:

The study was performed in accordance with OECD 111 on citric acid with purity of 99.5%.

The phthalate based buffer system detailed in the method guideline was determined to be unsuitable for testing as it caused significant interference during analysis. The alternative citrate buffer system was also unsuitable as it contained the species under investigation. Therefore both pH 4 and 7 sample solutions were initially prepared in an identical phosphate buffer solution and the nominal pH obtained using addition of 1 M hydrochloric acid and 2M sodium hydroxide solutions respectively.

Table 29: Type and composition of buffer solutions (specify kind of water if necessary)

pH	Type of buffer (final molarity)
4 and 7	Disodium hydrogen orthophosphate (anhydrous) (0.03 mol dm^{-3}) Potassium dihydrogen orthophosphate (0.02 mol dm^{-3}) Sodium chloride (0.02 mol dm^{-3})
9	Disodium tetraborate (0.01 mol dm^{-3}) Sodium chloride (0.02 mol dm^{-3})

Table 30: Description of test solution

Criteria	Details
Purity of water	Distilled, sterile water.
Preparation of test medium	Samples of test material were prepared in stoppered glass flasks at nominal concentration of 1.00 g/l in buffer solution. Solutions were shielded from light and maintained at the test temperature.
Test concentrations (mg a.i./L)	Stock solutions were diluted by a factor of 20 using 1% phosphoric acid (C0 = 0.05 g/l).
Temperature (°C)	30°C
Controls	None
Identity and concentration of co-solvent	None

Replicates	Duplicate

Table 31: Description of test system

Glassware	*Stoppered glass flask*
Other equipment	*HPLC system:* *Column: Synergi 4µ Hydro RP80A (250 x 4.6 mm id)* *Column temp: 30°C* *Mobile phase: 0.02M potassium dihydrogen orthophosphate: methanol (97:3 v/v), adjusted to pH 2.5 using phosphoric acid* *Flow rate: 1.0 ml/min* *UV detector: 220 nm* *Injecyion vol: 25 µl*
Method of sterilization	*Not specified*

Table 32: Hydrolysis of test compound, transformation products and reference substance, expressed as percentage of initial concentrations, at pH 4, pH 7 and pH 9.

pH 4	Sampling times (*hours*)	
	0	120
Parent compound (g/l)	1.02	1.02
Total % recovery	100	99.8
pH 7	Sampling times (*hours*)	
	0	120
Parent compound(g/l)	0.994	1.00
Total % recovery	100	101
pH 9	Sampling times (*hours*)	
	0	*120*
Parent compound (g/l)	0.999	1.01
Total % recovery	100	101

The preliminary test showed no dissipation at 50°C after 120 hours.

Conclusion

In accordance with the guideline, if less than 10% hydrolysis occurs after 5 days at 50°C, the equivalent half-life at 25°C is greater than 1 year.

ENVIRONMENTAL FATE

ADSORPTION/DESORPTION

4.1.4.3 O'Connor B and Mullee D (2006b)

Study reference:

O'Connor B and Mullee D, 2006, Determination of Adsorption Coefficient, Safepharm laboratories, 2089-008.

Detailed study summary and results:

The distribution coefficient (Kd) and organic carbon normalised adsorption coefficient (Koc) of citric acid in five different soil types were investigated in a GLP study. This study was performed by O'Connor and Mullee in 2006, Safepharm Laboratories Ltd., UK., conducted between 05 December 2005 and 10 March 2006, following OECD guideline No. 106 (2000) using citric acid, purity of 99.5%.

The study was divided in two parts: Tier 1, preliminary study and Tier 2, screening test where the equilibration time was reduced at 30 minutes instead of 24 hours in Tier 1. Guidance document SCP/KOC/002 in support of the related Council Directive 91/414/EEC stipulated that in the case of test materials unstable in the soil/solution systems, the favoured method to determine distribution coefficient and organic carbon content normalised distribution coefficient data was to retain the OECD 106 procedure but to reduce the adsorption equilibration period to only 30 minutes.

This was in line with the guidance document SCP/KOC/002 since no adsorption plateau was observed for citric acid in soil/solution systems attributed to biodegradation. Indeed, citric acid has been previously classified as inherently and readily biodegradable in study A.7.1.1.2.1 (see section 4.1.1.2 of this document).

Aliquots of soil were taken FEP/ETFE centrifuge tubes and 0.01M calcium chloride solution was added to each. The resulting mixtures were shaken at ambient temperature for a minimum of 12 hours, centrifuged and supernatants removed and filtered through 0.2 µm filters. For each soil type, an aliquot of 0.01M CaCl2 solution and an aliquot of the isolated soil aqueous phase were taken in triplicate FEP/ETFE centrifuge tubes. All test solutions were shaken in the dark at 25 ± 2°C for 24 hours or 30 minutes prior to analysis. Standard solution and sample solutions were analysed by high performance liquid chromatography (HPLC). Negligible biodegradation was anticipated over the reduced timescale of the amended procedure; and although the acidification of the samples was anticipated to quench any degradation during analysis, a vial cooler set at a nominal temperature of 4°C was used as a precautionary step to eliminate any biological activity.

Table 33: Soil characteristics in the adsorption/desorption experiment

Soil type	Sand (%)	Clay (%)	Organic C (%)	pH
OECD soil type 2	-	20-40	1.9	7.5
OECD soil type 3	-	15-25	3.2	6.4
OECD soil type 4	-	15-30	3.0	4.5
OECD soil type 5	-	<10-15	1.5	6.4
OECD soil type 7	-	<10	11.4	4.2

Table 34: Distribution coefficient (K_d, K_{oc}, 1/n and r for the five soil types)

Soil type	K_d	K_{oc}	Log K_{oc}
OECD soil type 2	8.97	472	2.67
OECD soil type 3	14.1	440	2.64

OECD soil type 4	10.9	363	2.56
OECD soil type 5	22.9	1.53×10^3	3.18
OECD soil type 7	8.68	76.2	1.88

Conclusion

According to the TGD part II chapter 3, substances with a Koc < 500 – 1000 l/kg are not likely sorbed to sediment (SETAC, 1993). The mean Koc values of citric acid ranging from 76.2 to 1530 l/kg, indicating that citric acid is moderately to slightly mobile in the five soils tested.

4.2 Bioaccumulation

Property	Value	Reference	Comment (e.g. measured or estimated)
Partition coefficient n-octanol/water	log Pow < -3.76 at +21°C	O'Connor BJ, 2006 Reliability 1	EC Method A8
	Log Kow values of -0.76±0.10 for H2A- and -0.21±0.11 for H3A were determined. In the presence of various surfactants Log Kow values obtained were: -0.45 to 0.13 for HA2-, 0.24 to 0.51 for H2A-, and 0.53 to 0.80 for H3A. (Citric acid monohydrate (analytical grade)	Unnamed, 1992 Reliability 2	REACH registration : Potentiometric method for determining the octanol-water partition coefficient (log P) for di- tri- and tetraprotic citric acid.
	Log Kow values of -1.61 to -1.80	Unnamed,1991 Reliability 2	REACH registration : Liquid-liquid extraction - flow injection analysis using coaxial segmentors to prevent phase separation.
	Log Kow value of -1.72	Verschueren K, 1996 Reliability 4	REACH registration : Handbook of Environmental Data on Organic Chemicals
	Log Kow of -1.72	Hansch C, Leo AJ, 1981 Reliability 4	REACH registration : Medchem Project. Issue No. 19 Claremont, CA: Pomona College. 1981
	Log Kow of -1.64	Avdeef A, 1997 Reliability 4	REACH registration : Seminar on Ionization & Lipophilicity. Log P values measured by pION Inc., Brookline, MA (A. Avdeef and C. Berger)
BCF	Citric acid bioaccumulation potential is based on its physico-chemical properties. Its BCF was estimated from log Pow (log Pow = -3.76) at 0.000127	OECD SIDS, 2000	Citric acid has a very low estimated BCF and has a low potential for bioaccumulation.

Conclusion

Based on the low Log Pow (log Pow = -3.76) and the requirement for citric acid uptake and metabolism for use in the Krebs Cycle, the substance will not bioaccumulate.

The active substance is a naturally occurring substance, which has high water solubility, is readily biodegradable and has a low log Pow. The active substance naturally occurs in all organisms and there is a mechanism for elimination of the substance via the Kreb's cycle. There is therefore negligible concern for bioaccumulation from the active substance.

4.2.1 Bioaccumulation test on fish

No experimental bioaccumulation studie on fish available. The BCF of citric acid was estimated from log Pow (log Pow = -3.76) at 0.000127.

Citric acid has a very low estimated BCF and has a low potential for bioaccumulation.

4.2.2 Bioaccumulation test with other organisms

No studies available.

4.3 Acute toxicity

4.3.1 Short-term toxicity to fish

4.3.1.1 [redacted] (2006)

Study reference:

[redacted]*., 2006, Citric Acid: Acute Toxicity to Rainbow Trout (Oncorhynchus mykiss), SafePharm Laboratories Ltd, SPL Project Number:* [redacted]

Detailed study summary and results:

Guideline	OECD 203
GLP	Yes
Deviations	No
Test material	Citric acid
Lot/Batch number	S504023
Purity	Not stated
Composition of Product	N/A
Further relevant properties	None stated
Method of analysis	HPLC using a Synergi Hydro RP 4 □ (250 x 4.6 mm id) column, a phosphate buffer:acetonitrile (95:5, v/v) mobile phase at a flow rate of 1 ml/min with an injection volume of 100 □l and UV-vis detection at 220 nm. The retention time was approximately 4 minutes.

Preparation of TS solution for poorly soluble or volatile test substances	Not required
Reference substance	No
Method of analysis for reference substance	N/A
Testing procedure	
Duration of the test	96 hours
Test parameter	Mortality
Sampling	The water temperature, pH and dissolved oxygen concentrations were recorded daily throughout the test. The measurements at 0 hours, and after each test media renewal at 24, 48 and 72 hours, represent those of the freshly prepared test preparations while the measurements taken prior to each test media renewal, and on termination of the test after 96 hours, represent those of the used or 24 hour old test preparations.
Monitoring of TS concentration	Yes - 0 hours (fresh media), 24 hours (old media) and 96 hours (old media)
Statistics	An estimate of the LC_{50} value was given by inspection of the mortality data.

Table 35: Dilution water

Criteria	Details
Source	Laboratory tap water
Alkalinity	Not stated
Hardness	100 mg/l as $CaCO_3$
pH	Not stated
Oxygen content	≥9.3 mg/l O_2
Conductance	Not stated
Holding water different from dilution water	No

Table 36: Test organisms

Criteria	**Details**
Species/strain	Juvenile rainbow trout (Oncorhynchus mykiss)
Source	Brow Well Fisheries Limited, Yorkshire, UK
Wild caught	Yes
Age/size	Length: 4.8 cm (sd = 0.3 cm) Weight: 1.77 g (sd = 0.56) Age: not stated
Kind of food	Commercial trout pellets

Amount of food	Not stated
Feeding frequency	Not stated
Pretreatment	12 days acclimation period
Feeding of animals during test	No

Table 37: Test system

Criteria	Details
Test type	Semistatic
Renewal of test solution	Daily renewal
Volume of test vessels	20 litres per vessel
Volume/animal	2 litres per fish
Number of animals/vessel	10
Number of vessels/ concentration	2
Test performed in closed vessels due to significant volatility of TS	No

Table 38: Test conditions

Criteria	Details
Test temperature	Range: 13.4 – 14.5 °C
Dissolved oxygen	Range: 8.0 – 10.1 mg O_2/l
pH	Range: 5.7 – 7.8
Adjustment of pH	No
Aeration of dilution water	Yes – test vessels were aerated via narrow bore glass tubes
Intensity of irradiation	Not stated
Photoperiod	16 h photoperiod daily

Table 39: Physico-chemical measurements

Nominal Concentration (mg/l)	Time (Hours)											
	0 Hours (Fresh Media)				24 Hours (Old Media)				24 Hours (Fresh Media)			
	pH	mg O_2/l	%ASV*	T°C	pH	mg O_2/l	%ASV*	T°C	pH	mg O_2/l	%ASV*	T°C
Control	7.4	8.0	76	13.4	7.8	9.3	92	14.5	7.4	9.0	87	13.5
100 R_1	5.8	8.1	79	13.5	7.4	9.6	93	14.4	5.8	8.9	86	13.4
100 R_2	5.8	8.1	77	13.4	7.4	9.7	94	14.4	5.8	9.0	87	13.5

Nominal Concentration (mg/l)	Time (Hours)											
	48 Hours (Old Media)				48 Hours (Fresh Media)				72 Hours (Old Media)			
	pH	mg O_2/l	%ASV*	T°C	pH	mg O_2/l	%ASV*	T°C	pH	mg O_2/l	%ASV*	T°C
Control	7.5	9.9	96	14.0	7.4	9.7	94	13.5	7.4	9.2	89	14.4
100 R_1	7.2	10.0	97	14.1	5.7	9.6	93	13.5	7.3	9.7	94	14.2
100 R_2	7.3	10.1	98	14.1	5.7	9.6	91	13.4	7.3	9.7	94	14.4

Nominal Concentration (mg/l)	Time (Hours)							
	72 Hours (Fresh Media)				96 Hours (Old Media)			
	pH	mg O_2/l	%ASV*	T°C	pH	mg O_2/l	%ASV*	T°C
Control	7.4	8.8	85	13.5	7.7	9.0	87	14.1
100 R_1	5.8	8.7	84	13.6	7.4	9.1	88	14.2
100 R_2	5.8	8.7	84	13.6	7.4	9.1	88	14.2

Conclusion

The results of the definitive test showed that the acute toxicity of citric acid to rainbow trout (Oncorhynchus mykiss) gave a 96-hour LC50, based on nominal test concentrations, greater than 100 mg/l.

4.3.2 Short-term toxicity to aquatic invertebrates

4.3.2.1 Mead C, Hill JWF (2006)

Study reference:

Mead C. and Hill J. W. F., 2006, Citric Acid: Acute Toxicity to Daphnia magna, SafePharm Laboratories Limited, SPL Project Number: 2189/0010

Detailed study summary and results:

Guideline study	Yes – OECD Guideline 202 (2004)
GLP	Yes
Deviations	No
	Materials and Methods
Test material	Citric acid
Lot/Batch number	*S504023*
Purity	*Not stated*
Composition of Product	*N/A*
Further relevant properties	*None stated*

Method of analysis	*HPLC using an external standard*
Reference substance	*Yes*
	Potassium dichromate
Method of analysis for reference substance	*Nominal test concentrations were used for EC_{50} analysis*
Testing procedure	
Duration of the test	*48 hours*
Test parameter	*Immobility*
Sampling	*Monitoring of Daphnia mobility performed at 24 and 48 hours after the beginning of the study.*
	For monitoring of test water concentrations, samples were taken at 0 and 48 hours and stored at approximately -20 °C for further analysis.
Monitoring of TS concentration	*Yes – at beginning and end of study*
Statistics	*Trimmed Spearman-Karber method (Hamilton* et al *1977) using the ToxCalc computer software package (ToxCalc 1999).*

Table 40: Dilution water

Criteria	Details
Source	*Reconstituted water*
Alkalinity	*Not specified*
Hardness	*250 mg/l as $CaCO_3$*
pH	7.8 ± 0.2
Ca / Mg ratio	*4:1*
Na / K ratio	*10:1*
Oxygen content	*Approximately air saturation value*
Conductance	<5 µS cm^{-1}
Holding water different from dilution water	*No*

Table 41: Test organisms

Criteria	Details
Strain	*1st instar Daphnia magna*
Source	*In house laboratory cultures*
Age	*< 24 hours*
Breeding method	*Parthenogenesis*
Kind of food	*Algal suspension (*Chlorella sp.*)*

Amount of food	*Not specified*
Feeding frequency	*Daily*
Pretreatment	*Not specified*
Feeding of animals during test	*No*

Table 42: Test system

Criteria	Details
Renewal of test solution	*None*
Volume of test vessels	*250 ml*
Volume/animal	*20 ml*
Number of animals/vessel	*10*
Number of vessels/ concentration	*2*
Test performed in closed vessels due to significant volatility of TS	*No*

Table 43: Test conditions

Criteria	Details
Test temperature	*20.0 – 20.3 °C*
Dissolved oxygen	*8.9 – 9.0 mg O_2/l*
pH	*Varied with test substance concentration:* *Control: 8.0* *10 mg/l: 6.1-6.4* *18 mg/l: 5.9-6.3* *32 mg/l: 5.6-5.9* *56 mg/l: 4.9* *100 mg/l: 4.1-4.2*
Adjustment of pH	*No*
Aeration of dilution water	*No*
Quality/Intensity of irradiation	*Not specified*
Photoperiod	*16 h photoperiod daily*

Results

Limit Test *no*

Concentration *0.10, 1.0, 10 and 100 mg/l*

Number/ percentage of animals showing adverse effects

Nominal concentration	Cumulative immobilised Daphnia (Initial population: 10 per replicate)

(mg/l)	24 hours	48 hours
Control	0	0
0.10	0	0
1.0	0	0
10	0	0
100	10	10

Nature of adverse effects: Immobilisation

Results test substance

Initial concentrations of test substance: 0, 10, 18, 32, 56 and 100 mg/l

Actual concentrations of test substance

Sample	Nominal Concentration (mg/l)	Concentration Found (mg/l)	Expressed as percent of nominal conc. (%)
0 hours	Control	<LOQ (0.48 mg/l)	-
	10	10.4	104
	18	20.9	116
	32	36.7	115
	56	60.6	108
	100	105	105
48 hours	Control	<LOQ	-
	10	5.7	57
	18	16.7	93
	32	31.5	99
	56	55.6	99
	100	102	102

Other effects: *None described*

Test with reference substance: *Performed*

Concentrations: *0, 0.32, 0.56, 1.0, 1.8 and 3.2 mg/l*

Results: *EC_{50} – >3.2 mg/l (3 hours)*

EC_{50} – 0.88 mg/l (24 hours)

EC_{50} – 0.60 mg/l (48 hours)

Applicant's Summary and conclusion

Results and discussion: *Throughout the test, all control and test concentrations were clear, colourless solutions.*

EC_{50}: 34 mg/l (48 hours) (30-38 mg/l – 95% confidence limits)

EC_{50}: 55 mg/l (24 hours) (48-62 mg/l – 95% confidence limits)

NOEC: 18 mg/l

Analysis of the test preparations at 0 and 48 hours showed measured test concentrations to range from 93% to 116% of nominal values with the exception of the 10 mg/l test sample at 48 hours which gave a

	measured concentration of 57% of nominal. *The decline in the measured concentration in the 10 mg/l test concentration as greater than that observed in the stability analyses. A reason for this could not be determined from the data, however, as this concentration was below the No Observed Effect Concentration it was considered justifiable to calculate the EC_{50} values in terms of the nominal test concentrations only.*
EC_{50}	*34 mg/l (48 hours) (30-38 mg/l – 95% confidence limits)*
Conclusion	*Analysis of the test preparations at 0 and 48 hours showed measured test concentrations to range from 93% to 116% of nominal values with the exception of the 10 mg/l test sample at 48 hours which gave a measured concentration 57% of nominal. The decline in the measured concentration in this test solution was greater than that observed in the stability analyses, given in appendix 1 of the report. A reason for this could not be determined from the data, however, as this concentration was below the NOEC it was considered justifiable to calculate the EC_{50} values in terms of the nominal test concentrations only.* *All other validity criteria can be considered as fulfilled*
Reliability	*1*
Deficiencies	No

4.3.3 Algal growth inhibition tests

4.3.3.1 McKenzie J, Vryenhoef H (2006)

Study reference:

McKenzie, J. and Vryenhoef, H., 2006, Citric Acid: Algal Inhibition Test, SafePharm Laboratories Limited, Report Number 2189/006.

Detailed study summary and results:

Guideline study	*Yes. OECD Guideline for Testing of Chemicals No. 201 "Alga, Growth Inhibition Test", Method C.3 of Commission Directive 92/69/EEC.*
GLP	*Yes*
Deviations	No
	Materials and Methods
Test material	*Citric Acid*
Lot/Batch number	*S504023*
Purity	*Not stated*
Method of analysis	*Performed on nominal concentrations*
Reference substance	*No*

Testing procedure

Culture medium

The culture medium was prepared using reverse osmosis deionised water and the pH adjusted to 7.5 ± 0.1 with 0.1N NaOH or HCl.

$NaNO_3$	25.5mg/l
$MgCl_2.6H_20$	12.164mg/l
$CaCl_2.2H_20$	4.41mg/l
$MgS0_4.7H_2O$	14.7mg/l
K_2HPO_4	1.044mg/l
$NaHCO_3$	15.0mg/l
H_3BO_3	0.1855mg/l
$MnCl_2.4HO$	0.415mg/l
$ZnCl_2$	0.00327mg/l
$FeCl_3.6H_2O$	0.159mg/l
$CoCl_2.6H_2O$	0.00143mg/l
$Na_2MoO4.2H_20$	0.00726mg/l
$CuCl_2.2H_20$	0.000012mg/l
$Na_2EDTA.2H_2O$	0.30mg/l
$Na_2SeO_3.5H_2O$	0.000010mg/l

Test organisms

Criteria	Details
Species	*Scenedesmus subspicatus*
Strain	*CCAP 276/20*
Source	*Dunstaffnage Marine Laboratory, Scotland*
Laboratory culture	*Yes*
Method of cultivation	
Pretreatment	*Acclimation*
Initial cell concentration	*5.96 x 10^3 cells/ml*

Test system

Criteria	Details
Volume of culture flasks	*250ml*
Culturing apparatus	*e.g. spectrophotometer, fluorimeter*
Light quality	
Procedure for suspending algae	*Describe briefly, e.g. shaking, stirring or aeration*
Number of vessels/ concentration	*3 vessels per concentration*
Test performed in closed vessels due to significant volatility of TS	*No*

Test conditions

Criteria	Details
Test temperature	*Give measurements conducted during test*
pH	*Give measurements conducted at start and end of test*
Aeration of dilution water	*No*
Light intensity	*Light intensity approximately 7000lux*
Photoperiod	*Duration of experiment*

Duration of the test: 72 Hours

Test parameter: Cell density inhibition

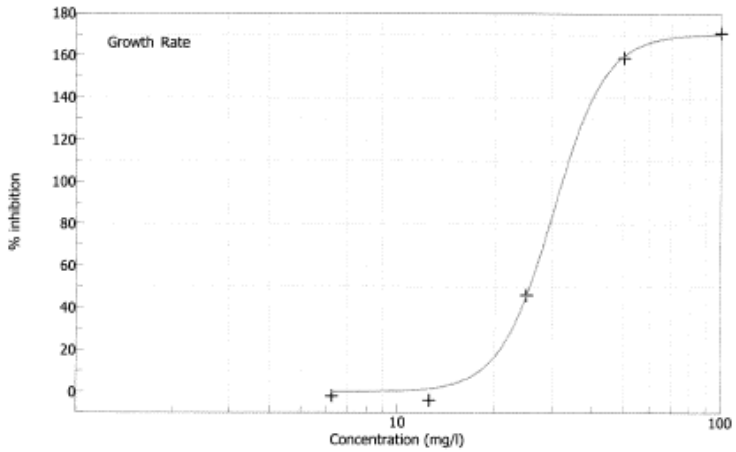
Sampling: Sampling occurred at 0, 24, 48 and 72 hours

Monitoring of TS concentration: Yes, test substance concentrations were measured at 0 and 72 hours

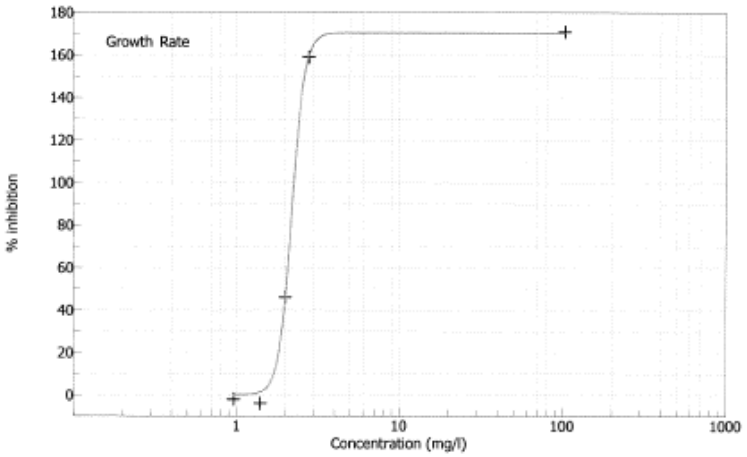
Statistics: The area under the growth curve is taken to be an index of growth. This was then compared to the area under the control curve, to give the

	percentage inhibition of growth. The geometric mean concentrations were calculated by multiplying the concentrations at the beginning and end of the test, and applying a square root to the result. Results
Limit Test	No
Concentration	
Number/ percentage of animals showing adverse effects	
Results test substance	*Non-entry field*
Initial concentrations of test substance	6.25, 12.5, 25, 50 and 100mg/l
Actual concentrations of test substance	5.75, 12.2, 25.2, 50.5 and 102mg/l
Growth curves	

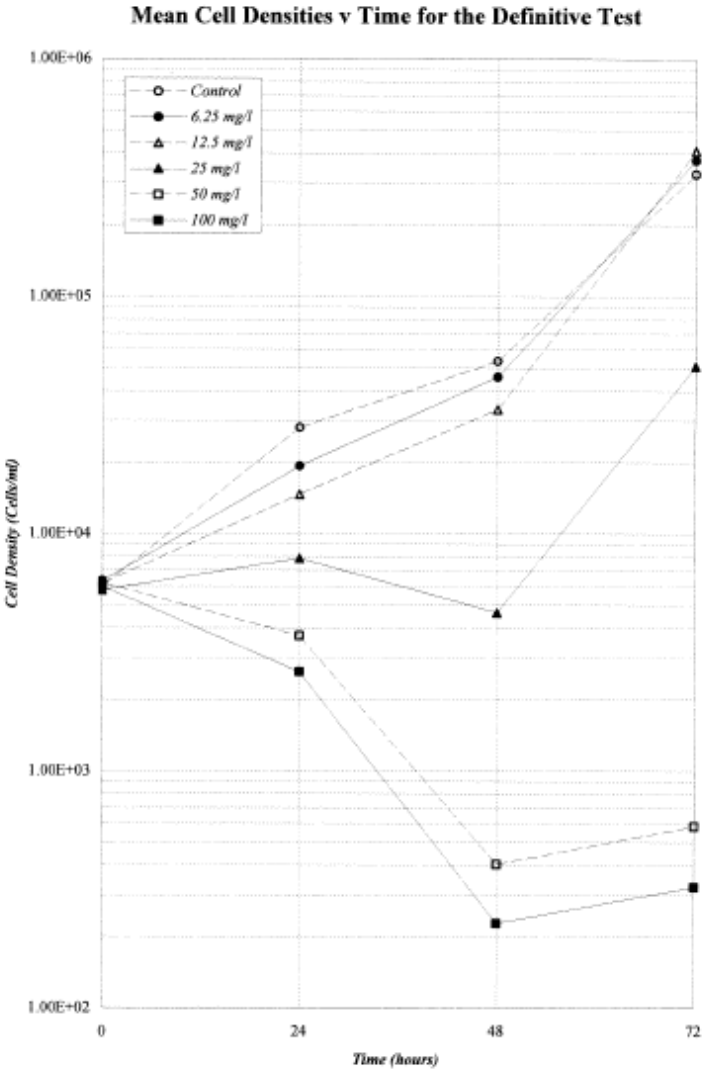
Inhibition of Growth Rate Based on Nominal Test Concentrations



Inhibition of Growth Rate Based on Geometric Mean Measured Test Concentrations



Concentration / response curve



Cell concentration data

Test-Substance Concentration (nominal) [mg/l]	Cell concentrations (mean values) [cells/ml] measured 0 h	24 h	48 h	72 h
6.25	6.30E+03	1.92E+04	4.58E+04	3.75E+05
12.5	6.15E+03	1.46E+04	3.34E+04	4.10E+05
25	5.76E+03	7.85E+03	4.63E+03	5.07E+04
50	6.19E+03	3.71E+03	4.03E+02	5.83E+02
100	5.98E+03	2.61E+03	2.27E+02	3.24E+02

Effect data (cell multiplication inhibition)

E_bC_{50}(72h) = 1.9mg/l

E_{r50}(0 - 72 h) = 2.0mg/l

NOE_rC = 1.4mg/l

Other observed effects

There was no reaction with the culture medium at the 100mg/l

concentration.

Results of controls

Cell concentrations (mean values) [cells/ml]			
measured			
0 h	24 h	48 h	72 h
5.96E+03	2.80E+04	5.33E+04	3.27E+05

Applicant's Summary and conclusion

Materials and methods	OECD Guideline for Testing of Chemicals No. 201 "Alga, Growth Inhibition Test", Method C.3 of Commission Directive 92/69/EEC
Results and discussion	An E_bC_{50} (72h) value of 23mg/l and an E_rC_{50} (0-72h) value of 26mg/l have been given. The No Observed Effect Concentration (NOEC) at 72 hours was 12.5mg/l. However, in cases where a decline in measured concentrations is observed, geometric mean measured concentrations are used for calculating EC_{50} values, therefore the results given below give a "worse case" analysis of the data.
NOE_rC	1.4mg/l
E_{r50}	2.0mg/l
E_bC_{50}	1.9mg/l
Conclusion	
Reliability	1
Deficiencies	No
Remark :	pH of each control and test flask was determined at initiation of the test and after 72h exposure. The pH values of the control cultures were observed to increase from 7.5 at 0h to 7.8 at 72h (< 1.5 pH units after 72h => within the limits given in the test Guidelines). The pH of the test cultures at 0 and 72h was observed to follow a concentration dependent decrease in pH with increasing test concentration, due to the presence of the test material (. This decline in pH may have contributed towards the observed toxicity of the test material.

4.3.4 *Lemna* sp. growth inhibition test

No data available.

4.4 Chronic toxicity

4.4.1 Fish early-life stage (FELS) toxicity test

No data available.

4.4.2 Fish short-term toxicity test on embryo and sac-fry stages

No data available.

4.4.3 Aquatic Toxicity – Fish, juvenile growth test

No data available.

4.4.4 Chronic toxicity to aquatic invertebrates

No data available.

4.4.5 Chronic toxicity to algae or aquatic plants

[See short-term toxicity]

4.5 Acute and/or chronic toxicity to other aquatic organisms

4.5.1 OECD TG 209: Activated Sludge, Respiration Inhibition Test

4.5.1.1 Clarke N (2006)

Study reference:

Clarke, N (2006) Assessment of the Inhibitory Effect on the Respiration of Activated Sewage Sludge. SafePharm Laboratories. SPL Project Number: 2189/007.

Detailed study summary and results:

	Guidelines and Quality Assurance
Guideline study	OECD (1984) 209, EEC Commission Directive 87/302/EEC and US EPA OPPTS 850.6800
GLP	Yes
Deviations	No
	Materials and Methods
Test material	Citric acid
Lot/Batch number	S504023
Purity	Not stated
Further relevant properties	Store at room temperature in the dark
Method of analysis	Karl-Fischer method
Reference substance	3,5-dichlorophenol (Sigma-Aldrich Batch No. 15809KI023)

Method of analysis for reference substance	Reference substance has batch number from Sigma-Aldrich.
Testing procedure	Non-entry field
Culture medium	A synthetic sewage of the following composition was used. 16g Peptone, 11g Meat extract, 3g Urea, 0.7g NaCl, 0.4g $CaCl_2.H_2O$, 0.2g $MgSO_4.7H_2O$, 2.8g K_2HPO_4 Dissolved in litre of water with the aid of ultrasonication.
Inoculum / test organism	See table below
Test system	See table below
Test conditions	See table below
Duration of the test	3 hours contact time
Test parameter	Respiration inhibition
Analytical parameter	Oxygen consumption
Sampling	After 30 minutes contact time and 3 hours contact time
Monitoring of TS concentration	No
Controls	2 controls without test substance were prepared

Table 44: Inoculum / Test organism

Criteria	Details
Nature	*Mixed population of activated sewage sludge micro-organisms*
Species	*Predominantly domestic sewage*
Strain	*n/a*
Source	*Severn Trent Water Plc sewage treatment plant UK*
Sampling site	*n/a*
Laboratory culture	*No*
Method of cultivation	*n/a*
Preparation of inoculum for exposure	*Kept at 21°C and filtered.*
Pretreatment	*None*
Initial cell concentration	*Suspended solids was equal to 4.0g/l prior to use*

Table 45: Test system

Criteria	Details
Culturing apparatus	*500ml conical flask.*

Number of culture flasks/concentration	*1 flask per concentration*
Aeration device	*Narrow bore glass tube (0.5-1 litre per minute).*
Measuring equipment	*BOD bottle and Yellow Springs dissolved oxygen meter fitted with a BOD probe.*
Test performed in closed vessels due to significant volatility of TS	*No*

Table 46: Test conditions

Criteria	Details
Test temperature	*21°C*
pH	*Was not controlled during test but observed: 7.5*
Aeration of dilution water	*0.5-1 litre per minute (continuous aeration)*
Suspended solids concentration	*4.0 g/l*
Total hardness	*100 mg/l as $CaCO_3$*

Statistics	Percentage inhibition values were plotted against concentration, a line fitted using Xlfit3 software package (IDBS 2002).
	Results
Preliminary test	Performed
Concentration	0.10, 1.0, 10, 100, 1000 mg/l.
Effect data	Significant inhibition of respiration was observed at 1000 mg/l, however there was no significant effects on respiration at 0.10, 1, 10, and 100 mg/l.
Results test substance	Non-entry field
Initial concentrations of test substance	100, 180, 320, 560 and 1000 mg/l
Actual concentrations of test substance	n/a
Growth curves	n/a
Cell concentration data	n/a
Concentration/ response curve	Plot of the percent inhibition vs. concentration of test substance
Effect data	3-Hour EC_{50} of 370 mg/l. The NOEC after 3 hours exposure was 180 mg/l.
Other observed effects	None
Results of controls	2 controls without test item in gave O_2 consumption rates of 0.63 and 0.60 mg O_2/l/min after 30 minutes and 0.56 and 0.51 mg O_2/l/min after 3 hours.Variation in the 2 controls were ± 2% and ± 5% after 30 minutes and 3 hours respectively.
Test with reference substance	Performed.
Concentrations	3.2 and 32 mg/l
Results	14 mg/l and 10 mg/l after 30 minutes and 3 hours respectively
	Applicant's Summary and conclusion
Materials and methods	At time “0” 16ml of synthetic sewage was diluted to 300ml with water and 200ml of inoculum added in a 500ml conical flask (first control). The mixture was aerated with compressed air via narrow bore glass tubes at a rate of approximately 0.5 - 1 litre per minute. Thereafter, at

	15 minute intervals the procedure was repeated with appropriate amounts of the reference material being added. As each vessel reached 30 minutes contact time an aliquot was removed from the conical flask and poured into the measuring vessel (250ml darkened glass Biological Oxygen Demand (BOD) bottle) and the rate of respiration measured using a Yellow Springs dissolved oxygen meter fitted with a BOD probe. The contents of the measuring vessel were stirred constantly by magnetic stirrer. The rate of respiration of each flask was measured over the linear potion of the oxygen consumption trace (between approximately 8.8mg O_2/l and 1.5mg O_2/l). After measurement the contents of the BOD bottle were returned to the test vessel. This procedure was repeated after 3 hours contact time. The test was conducted under normal laboratory lighting in a temperature controlled room at 21°C.
Results and discussion	The effect of the test material on the respiration of activated sewage sludge micro-organisms gave a 3-Hour EC_{50} of 370mg/l. The No Observed Effect Concentration (NOEC) after 3 hours was 180mg/l.
EC_{20}	
EC_{50}	370mg/l after 3 hours contact.
EC_{80}	
Conclusion	
Reliability	1

Attachment 1: Citric acid CAR Section A3

Section A3 **Physical and Chemical Properties of Active Substance**

Subsection (Annex Point)	Method	Purity/ Specification	Results Give also data on test pressure, temperature, pH and concentration range if necessary	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
3.1 Melting point, boiling point, relative density (IIA3.1)						0		
3.1.1 Melting point								
Melting pt. 1	EC Method A1	99.5%	151°C		Yes	1	O'Connor, B (2006) Safepharm Laboratories Study No 2189/001	x
Melting pt. 2	No data	Anhydrous form	153°C	The active substance is a naturally occurring substance derived from a food source. There will therefore be variability in the active substance when sourced from different producers. However, the specification is for purity of 99.5% or greater. It is therefore	No data	3	Daubert, TE et al (1995) Physical and thermodynamic properties of pure chemicals. Data Compliat. Taylor and Francis	x

Section A3	**Physical and Chemical Properties of Active Substance**							
Subsection (Annex Point)	**Method**	**Purity/ Specification**	**Results** Give also data on test pressure, temperature, pH and concentration range if necessary	**Remarks/ Justification**	**GLP (Y/N)**	**Reliability**	**Reference**	**Official use only**
				considered that the physicochemical characteristics of the pure form will be applicable to this specification of the active substance and that further physico-chemical studies are not justified.				
3.1.2 Boiling point Boiling pt.	EC Method A2	99.5%	Decomposed from ca. 171°C at 102.83 kPa prior to boiling		Yes	1	O'Connor, B (2006) Safepharm Laboratories Study No 2189/001	x
3.1.3 Bulk density/ relative density Bulk/rel. density 1	EC Method A3	99.5%	Relative density: 1.65 E+03 kg/m^3		Yes	1	O'Connor, B (2006) Safepharm Laboratories Study No 2189/001	x

Section A3	**Physical and Chemical Properties of Active Substance**							
Subsection (Annex Point)	**Method**	**Purity/ Specification**	**Results** Give also data on test pressure, temperature, pH and concentration range if necessary	**Remarks/ Justification**	**GLP (Y/N)**	**Reliability**	**Reference**	**Official use only**
Bulk/rel. density 2	DIN 53912		Relative density: 1.665 kg/m^3	The active substance is a naturally occurring substance derived from a food source. There will therefore be variability in the active substance when sourced from different producers. However, the specification is for purity of 99.5% or greater. It is therefore considered that the physicochemical characteristics of the pure form will be applicable to this specification of the active substance and that further physico-chemical studies are not	No data	4	CRC Handbook of Chemistry and Physics, 70th Edn	x

Section A3	**Physical and Chemical Properties of Active Substance**							
Subsection (Annex Point)	**Method**	**Purity/ Specification**	**Results** Give also data on test pressure, temperature, pH and concentration range if necessary	**Remarks/ Justification**	**GLP (Y/N)**	**Reliability**	**Reference**	**Official use only**
				justified.				
3.2 Vapour pressure (IIA3.2) Vapour pressure 1	OECD 104	99.5%	**Result:** 1.6 E-10 Pa **Temp.**: 25°C		Yes	1	O'Connor, B (2006) Safepharm Laboratories Study No 2189/001	x
3.2.1 Henry´s Law Constant (Pt. I-A3.2)	Calculated		**Result:** 4.398E-14 $Pa.m^3/mole$					x
3.3 Appearance (IIA3.3)								
3.3.1 Physical state			Crystalline powder		No data	4	OECD SIDS dossier	
3.3.2 Colour			White or colourless		No data	4	OECD SIDS dossier	
3.3.3 Odour				No data				
3.4 Absorption spectra (IIA3.4)	Not applicable	99.5%			Yes	1	O'Connor, B (2006) Safepharm Laboratories Study No 2189/001	x
UV/VIS			No significant absorption at 290 nm					
IR			Consistent with proposed structure					

Section A3	**Physical and Chemical Properties of Active Substance**							
Subsection (Annex Point)	**Method**	**Purity/ Specification**	**Results** Give also data on test pressure, temperature, pH and concentration range if necessary	**Remarks/ Justification**	**GLP (Y/N)**	**Reliability**	**Reference**	**Official use only**
NMR **MS**			Consistent with proposed structure Consistent with proposed structure					
3.5 Solubility in water (IIA3.5) Water solubility	*including effects of pH (5-9)* EC Method A6	99.5%	**result:** 65 – 69.9% w/w **temperature:** 10, 20 and 30°C **pH:** Substance ionises at pH 5-9		Yes	1	O'Connor, B (2006) Safepharm Laboratories Study No 2189/001	x
3.6 Dissociation constant (-)	OECD 112	99.5%	pK_1 =3.01 pK_2 = 4.50 pK_3 = 5.87		Yes	1	O'Connor, B (2006) Safepharm Laboratories Study No 2189/001	x
	Various methods.	Not stated	25°C pK_1 =2.63 – 3.44 pK_2 =4.11 – 5.02 pK_3 =5.34 – 6.55	The active substance is a naturally occurring substance derived from a food source. There will therefore be variability in the active substance when sourced	No data	2	Serjeant EP, Dempsey B. Ionisation constants of organic acids in aqueous solution. IUPAC Chemical	

Section A3	**Physical and Chemical Properties of Active Substance**							
Subsection (Annex Point)	**Method**	**Purity/ Specification**	**Results** Give also data on test pressure, temperature, pH and concentration range if necessary	**Remarks/ Justification**	**GLP (Y/N)**	**Reliability**	**Reference**	**Official use only**
				from different producers. However, the specification is for purity of 99.5% or greater. It is therefore considered that the physicochemical characteristics of the pure form will be applicable to this specification of the active substance and that further physico-chemical studies are not justified.			Data Series – No. 23. Pergamon Press	
3.7 Solubility in organic solvents, including the effect of temperature on solubility (IIIA3.1)	CIPAC Method 181 and EC Method A6	99.7 – 100.3%	Hexane ≤ 7.84 E-05 g/L Diethyl ether = 7.34 g/L Methanol > 250 g/L		Y	1	Fox JM and White DF (2010) Citric acid: Determination of solubility in organic solvents.	

Section A3	**Physical and Chemical Properties of Active Substance**							
Subsection (Annex Point)	**Method**	**Purity/ Specification**	**Results** Give also data on test pressure, temperature, pH and concentration range if necessary	**Remarks/ Justification**	**GLP (Y/N)**	**Reliability**	**Reference**	**Official use only**
							Harlan laboratories No: 2189/0011	
3.8 Stability in organic solvents used in b.p. and identity of relevant breakdown products (IIIA3.2)				The active substance will not be used in organic solvents in the biocidal product.				

Section A3		**Physical and Chemical Properties of Active Substance**							
Subsection (Annex Point)		**Method**	**Purity/ Specification**	**Results** Give also data on test pressure, temperature, pH and concentration range if necessary	**Remarks/ Justification**	**GLP (Y/N)**	**Reliability**	**Reference**	**Official use only**
3.9	**Partition coefficient n-octanol/water (IIA3.6)**	*including effects of pH (5-9)*							
	log Pow 1	EC Method A8	99.5%	**result:** Pow **≤** 1.75 E-04, log Pow ≤ -3.76 **temperature:** 21°C **pH:** Substance ionises at all pH		Yes	1	O'Connor, B (2006) Safepharm Laboratories Study No 2189/001	x
	log Pow 2	Similar to shake flask method	Not stated	**result:** 0.019 **temperature:** 20°C **pH:**		No GLP not compulsory at time of study	2	COLLANDER R (1951) THE PARTITION OF ORGANIC COMPOUNDS BETWEEN HIGHER ALCOHOLS AND WATER. ACTA CHEM. SCAND. V5:771-780	X

Section A3		**Physical and Chemical Properties of Active Substance**							
Subsection (Annex Point)		**Method**	**Purity/ Specification**	**Results** Give also data on test pressure, temperature, pH and concentration range if necessary	**Remarks/ Justification**	**GLP (Y/N)**	**Reliability**	**Reference**	**Official use only**
3.10	**Thermal stability, identity of relevant breakdown products (IIA3.7)**	OECD 113	99.5%	Thermally stable and stable in air up to at least 150°C		Yes	1	O'Connor, B (2006) Safepharm Laboratories Study No 2189/001	x
3.11	**Flammability, including auto-flammability and identity of combustion products (IIA3.8)**	EC Method A10	99.5%	Not highly flammable		Yes	1	Tremain, S (2006) Safepharm Laboratories Study No 2189/003	x
	Relative Self-Ignition Temperature	EC Method A16	99.5%	None below melting temperature		Yes	1	Tremain, S (2006) Safepharm Laboratories Study No 2189/003	x
3.12	**Flash-point (IIA3.9)** Flash-point 1 Flash point 2				Test not applicable to solids				

Section A3	**Physical and Chemical Properties of Active Substance**							
Subsection (Annex Point)	**Method**	**Purity/ Specification**	**Results** Give also data on test pressure, temperature, pH and concentration range if necessary	**Remarks/ Justification**	**GLP (Y/N)**	**Reliability**	**Reference**	**Official use only**
3.13 Surface tension (IIA3.10) Surface tension	EC Method A5	99.5%	**result:** 73.6 mN/m for a 1.01g/l solution **temperature:** 21°C		Yes	1	O'Connor, B (2006) Safepharm Laboratories Study No 2189/001	x
3.14 Viscosity (-)			**result: temperature:**	Test not applicable to solids				
3.15 Explosive properties (IIA3.11)	EC Method A14	99.5%	Predicted to be negative based upon structure		Yes	1	Tremain, S (2006) Safepharm Laboratories Study No 2189/003	x
3.16 Oxidizing properties (IIA3.12)	EC Method A17	99.5%	Predicted to be negative based upon structure		Yes	1	Tremain, S (2006) Safepharm Laboratories Study No 2189/003	x
3.17 Reactivity towards container material (IIA3.13)								