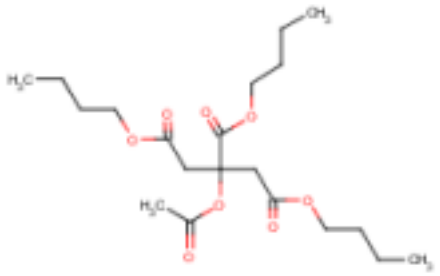
ACETYL TRIBUTYL CITRATE

SYNONYMS

2-Acetoxy-1,2,3-propanetricarboxylic acid tributyl ester
2-(Acetyloxy)-1,2,3-propanetricarboxylic acid tributyl ester
2-Acetyl tributyl citrate
Acetyl tributyl citrate
Acetylcitric acid, tributyl ester
Blo-trol
Citric acid, tributyl ester, acetate
Citroflex A
Citroflex A 4
Tributyl 2-(acetyloxy)-1,2,3-propanetricarboxylate
Tributyl 2-acetoxy-1,2,3-propanetricarboxylate
Tributyl O-acetylcitrate
Tributyl acetyl citrate
Tributyl acetylcitrate
Tributyl citrate acetate

CHEMICAL STRUCTURE



CHEMICAL FORMULA

$C_{20}H_{34}O_8$

IDENTIFIER DETAILS

CAS Number	:	77-90-7
CoE Number	:	-
FEMA	:	3080
EINECS Number	:	201-067-0
E Number	:	-

SPECIFICATIONS

Melting Point: -76.00 to -75.00 °C at 760.00 mm Hg

Boiling point: 172.00 to 173.00 °C at 1.00 mm Hg

STATUS IN FOOD, TOBACCO AND DRUG LAWS

CoE limits:

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

Acceptable Daily Intake:

ADI (mg/kg)	ADI Set by	Date Set	Comments
Acceptable	JECFA	1999	No safety concern at current levels of intake

FDA Status [CFR 21]:

Section Number	Comments
175.515	Synthetic flavouring substances and adjuvants

HUMAN EXPOSURE

Natural Occurrence: Not reported found in nature (Fenaroli, 2005).

Reported Uses: ATBC is used in alcoholic beverages at 1 ppm (Fenaroli, 2005).

Sources other than in foods : ATBC is used as a plasticizer with aqueous- and solvent-based polymers, including acrylic, methacrylic, ethyl cellulose, hydroxypropyl methyl cellulose, nitrocellulose, vinyl acetate, vinyl chloride, vinyl pyrrolidone, vinylidene chloride, and urethane polymer systems. ATBC is also used in the following applications: ***Medical plastics*** : Aqueous pharmaceutical coatings; extra-corporeal tubing. ***Food contact products*** : Food wraps and films; beverage tubing; crown liners; food cont ainers; tinplate lubricant; aluminum foil coatings. ***Cellulosics***: Nitrocellulose-based explosives/propellants. ***Other industrial uses*** : Children 's toys; animal ear tags; ink formulations; adhesives; pesticide inerts (EPA 2003).

TOXICITY DATA

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

In Vivo Toxicity Status

Species	Test Type	Route	Reported Dosage
Mouse	LD_{50}	I.P.	> 4 g/kg (Meyers et al., 1964)
Mouse	LD_{50}	I.P.	4 g/kg (Doull et al., 1980)
Rat	LD_{50}	Oral	31.4 g/kg (Doull et al., 1980)
Rat	LD_{50}	Oral	> 30 g/kg
Cat	LD_{50}	Oral	> 50 g/kg (BIBRA, 1989)

Groups of 22 rats (10 male and 12 female) were given actely tributyl citrate at 5 % (2.5 g/kg bw/day) or 10 % in the diet for 6 – 8 weeks. There was no deleterious effect on growth at 5 % but 10 % caused frequent diarrhoea and markedly depressed growth. Microscopic examination of the major organs revealed no cellular abnormalities (BIBRA, 1989).

Five rats (strain and weight not stated) were fed 10 to 30 mL/kg acetyl tributyl citrate and then observed for up to 3 weeks. None of th e animals died and signs of systemic toxicity were not observed (Johnson, 2002).

A single dose of acetyl tributyl citrate (30 to 50 mL/kg) was administered by stomach tube to each of four fasted cats and animals were observed for 2 months. Two additional cats served as controls. Signs of nausea were observed in test animals, and, within a few hours of dosing, diarrhoea (oozing of oily material) was noted. The diarrhoea subsided within 24 hours of dosing. The behaviour and general appearance of animals indi cated systemic toxicity. Two cats dosed with 50 mL/kg were used for haematological evaluations and no effects on the following blood parameters were found: blood cell counts, haemoglobin, sugar, non -protein nitrogen, or creatinine. Results from urinalyses indicate no abnormalities in specific gravity, albumin, sugar, pH, or microscopic formed elements (Johnson, 2002).

Acetyl tributyl citrate administrated by IP in graded doses (in 3 % acacia) to Swiss albino mic e, failed to induce rapid loss of the rig hting reflex in mice at doses slightly greater than 400 mg/kg, but did cause an increase in respiratory rate. T he increase in respiratory rate was frequently accompanied by clonic convulsions. Writhing was also observed during the first 10 minutes post injection. Similar effects were observed in Wistar rats dosed intraperitoneally with acetyl tributyl citrate . A marked increase in respiratory rate and motor activity was observed in rabbits administered intravenously with 100 mg/kg acetyl tributyl citrate (Johnson, 2002).

In a 14-day subacute study, mice were administered 900 mg/kg by intraperitoneal injection of acetyltributyl citrate. There were no significant effects in weight gain or haematology except for decreased red blood cell count (Clayton et al., 1993).

Acetyl tributyl citrate (in 3 % acacia) was evaluated using groups of 20 Swiss albino mice (weights = 16 to 20 g). Acetyl tributyl citrate (900 mg/kg) was injected intraperitoneally daily for 14 days. Acetyl tributyl citrate reduced body weight gain (significant within 95 % confidence limits at day 7). Compared to controls acetyl tributyl citrate caused a significant (95 % confidence) decrease in the erythrocyte count and haemoglobin concentration. Pathological changes were not observed in the liver, lungs, or kidneys (Johnson, 2002).

Acetyl tributyl citrate was fed to groups of four 21-day-old rats (males and females; strain not stated) for 6 weeks. Two groups were fed test substance concentrations of 5 % and 10 %, respectively, for 6 weeks. A third group served as the control. No deleterious effects on growth were noted following the ingestion of the 5 % diet. Feeding of the 10 % diet resulted in growth reduction, which could have resulted from the diarrhoea (Johnson, 2002).

Acetyl tributyl citrate was injected into two rabbits (IP dose = 450 mg/kg) daily for 14 days and into two other rabbits (IP dose = 900 mg/kg) daily for 7 days. A decrease in red blood cell count (ranging from 0.5 to 2.5 million) and a corresponding decrease in haemoglobin concentration were noted in all animals. Bone marrow smears indicated no evidence of aplastic anaemia (Johnson, 2002).

Acetyl tributyl citrate was evaluated using three groups of male and female rats (number of animals, weights, and strain not stated). The three groups were fed 100, 300 and 1000 mg/kg body weight, respectively, for 90 days. A fourth group served as the control. Liver enlargement in rats (both sexes) of the 1000 mg/kg dose group and in the male rats of the 300 mg/kg dose group was the only treatment-related change considered important. Liver enlargement was not accompanied by any evidence of hepatic biochemical or histopathological changes (Johnson, 2002).

Mice were given daily IP injections of 0.9 g/kg bw of acetyl tributyl citrate for 14 days. Weight gain was inhibited and there was a fall in the red blood cell count and haemoglobin levels, but no cellular changes were observed microscopically in the liver, lungs or kidneys (Meyers et al., 1964).

Summary of Repeated Dose Toxicity Studies

Species; Sex	Route	No. of Treatment Groups / No. of Animals per Group	Duration	NOAEL (mg/kg bw/day)
Rat; M & F	Oral (feed)	2 / 4	6 weeks	5%

Rat; M & F	Oral (feed)	2 / 4	8 weeks	10%
Cat; NS	Oral (gavage)	1 / 2	2 months	5250 a
Mouse; NS	I.P. injection	1 / 5	14 days	<900
Rat; M & F	Oral (feed)	3 / 10	14 days	<1000
Rat; M & F	Oral (feed)	3 / 40	90 days	300
Rat; NS	Oral (feed)	3 / 20	2 years	100
Rat; M & F	Oral (feed)	3 / 50	In utero exposure phase + 90 days	M = 100 F = 300

M = Male; F = Female NS = Not statistical differences. Assumes a specific gravity of 1.05 (EPA 2003)

Carcinogenicity and Mutagenicity

Three groups of 1 month old Sherman rats (20 rats per group) were fed diets containing 200, 2,000 or 20,00 0 ppm acetyl tributyl citra te, for 2 years. Compared to the control group, transient reduction of the growth rate was noted in all three test groups during week 5 to 15 of the study; however, the difference was not statistically significant. The difference in mortality between the test and control groups was also not statistically significant. Twelve test animals and 8 control animals died spontaneously. Differences in behavio ur between test and control animals were not observed and the incidence of diarrhoea in test animals was no greater than that noted for controls. At necropsy, inflammatory disease of the lungs was the most frequent finding, but pathological findings between test and control groups wer e not statistically significant. The endocrine organs were free of abnormalities (Johnson, 2002).

Groups of 5 male rats were treated with 800 or 2000 mg/kg acetyl tributyl citrate in corn oil by oral gavage at a dose volume of 10 mL/kg. These concentrations did not induce unscheduled DNA synthesis in fresh ly prepared primary cultures of hepatocytes from the rat livers (EPA, 2003).

Dermal Toxicity

The skin irritation potential of acetyl tributyl citrate was evaluated using 59 men and women (age range = 21 - 60 years), all of whom had history of diabetes, psoriasis, or active dermatoses. Occlusive patches moistened with 0.4 mL of acetyl tributyl citrate were applied to the upper arm of each subject 3 times a week for 3 consecutive weeks. Each patch was removed 24 hours post application. Induction reactions were scored prior to patch applications (second through ninth visits) and at the time of the tenth visit. Duplicate challenge of the test material was made after a t wo-week non-treatment period. One challenge patch was applied to the original test site and another to an adjacent site. Challenge reactions were scored at 48 and 96 hours post application. Acetyl tributyl citrate was shown to be non-irritating to the skin, and reactions suggestive of contact sensitization were not observed during the study (Johnson, 2002).

Acetyl tributyl citrate caused moderate erythema of the eye in two out three male albino rabbits within 20 minutes post instillation 0.1 mL was instilled into

the left conjunctival sac of each animal. Moderate. The erythema persisted to 3 hours post instillation and subsided in one of the animals after 5 hours. At 24 hours, the moderate erythema observed in one rabbit was slightly increased, whereas the other two rabbits were classified as negative for ocular irritation. All eyes were essentially negative at 48 and 72 hours.
Acetyl tributyl citrate applied at 100 % induced barely perceptible erythema of the skin in one of four guinea pigs at 24 and 48 hours after patch removal. Reactions were not observed at lower test concentrations (Johnson, 2002).

The application of undiluted acetyl tributyl citrate to the skin of guinea pigs did not induce pathological reactions. At the end of the experiment, application of the test substance in fractions of 1/10 and 1/20 of the maximum 2,500 and 500 mg/kg dose and 1/10 and 1/20 of the threshold and permitted dose of 12,500 mg/kg did not produce significant effects. However, the periodic application of acetyl tributyl citrate (250 and 500 mg/kg doses) during the experiment caused loss of body weight, a decr ease in cerebral perfusion pressure, and an increase in the liver weight coefficient (Johnson, 2002).

Corneal reflex action was temporarily abolished when three drops of a 5 % suspension of acetyl tributyl citrate in a 3 % gum acacia medium was instilled in the rabbit eye (Meyers et al., 1964).

Reproductive and Developmental Toxicity

Parental (F0) animals (25 Hans Wistar rats/sex/group) were exposed to acetyl tributyl citrate continuously in the diet at targe t doses of 0, 100, 300 and 1000 mg/kg/day for 4 week s before pairing and mating. F0 females were treated t hroughout gestation, littering and lactation until the litters were weaned on lactation day 21. The offspring (F1) were exposed to the test material *in utero* and from birth until the start of the 13-wk study (when the animals were approximately 4 weeks of age). During the 13-wk study, the F1 animals (20/sex/group) were administered acetyl tributyl citrate in the diet at the same target doses as the parental animals. In the 13-wk toxicity phase of the study, administration of acetyl tributyl citrate via the diet at doses as high as 1000 mg/kg/day to rats that had already received direct and indirect exposure to the test material from before conception did not produce any marked toxicity.

Treatment at 1000 mg/kg/day resulted in a slight reduction in body weight gain in both sexes, which was considered to be a nonspecific indicator of toxicity. Liver weights were increased and hepatic hypertrophy occurred at 1000 mg/kg/day in both sexes. Hepatic hype rtrophy resulting from an induction of metabolizing enzymes as an adaptive response to treatment is a common finding following administration of high doses of xenobiotics, and is not considered to be toxicologically significant. Slight variations in urinar y composition and in plasma electrolyte concentrations suggested an effect on renal func tion at the higher dose levels which was considered to be an adaptative change to the excretion of high levels of the test material and/or metabolites and is not consid ered to be of any toxicological significance. The NOAELs for systemic toxicity for in this 13 -wk toxicity study with an in utero

exposure phase were considered to be 100 mg/kg/day for males and 300 mg/kg/day for females (EPA, 2003).

ATBC was evaluated for developmental toxicity potential in 12-month studies conducted with rats and mice. Groups of rats and mice were provided feed which contained a milk solution of the test substance (ATBC) at doses of 50 and 250 mg/kg bw/day for 12 months. A third group served as a control. In the ninth month of the study, a cross-mating of the animals was performed, male gonads were evaluated and embryotoxic effects were examined. The following indicators of embryotoxic effects were evaluated: early and late embryonic death (determined by examining the numbers of corpora lutea and implantation sites); and the number of normal, resorptive and deformed tissues. The length of the newborns was measured as was the size and weight of the placenta. Physiological development of the progeny also was evaluated by the following parameters: ear openings, eye openings, appearance of body hair and teeth, behavior and body weight. In both species there were no effects of treatment noted at doses of 50 mg/kg bw/day. ATBC had no significant effects in rats or mice on male gonads, and the spermatogenesis index in animals of the 250 mg/kg bw/day group was similar to controls. Increases in body weight and length of the progeny and placental weight were observed in the 250 mg/kg bw/day dose group. There were no differences between groups in the fertility rate and number of animals born per pregnant female. The physiological development (i.e. eye and ear opening, and body fur and incisor appearance), behavior and body weight of the progeny also were unaffected by treatment. The developmental toxicity NOAEL in both studies was 250 mg/kg bw/day (EPA 2003)

Also regarding developmental toxicity, no developmental effects were observed at dose levels as high as 1000 mg/kg bw/day in a two-generation reproduction study nor in a 13-week toxicity study with an in utero exposure phase. ATBC is rapidly and extensively absorbed, and then rapidly metabolized and virtually completely excreted by the rat. The metabolites that have been positively identified in the urine of rats (acetyl citrate, monobutyl citrate, acetyl monobutyl citrate, dibutyl citrate and two isomers of acetyl dibutyl citrate) have been demonstrated to undergo rapid clearance from the body and are not suspected to be developmental toxicants. Also, other ATBC metabolites, acetic acid, citric acid, butyric acid, tributyl citrate and butanol, do not pose a concern for developmental toxicity (EPA 2003)

ATBC was tested for androgenic and oestrogenic activity by means of estrogen receptor (ER) and androgen receptor (AR) competitive ligand-binding assays. Further, in vivo experiments, using ovariectomized Sprague-Dawley rats were observed for uterine wet weight change, uterine endometrium hyperplasia and vaginal mucosal cornification, following administration of ATBC extract orally (at 0.5 or 500 mg/kg) or subcutaneously (at 0.5 or 100 mg/kg). No significant response or change was observed with ATBC, either in vitro or in vivo. The results indicated that ATBC did not exerted any sex-hormonal activity (Ohta M, Oshima S *et al.,* 2003).

Behavioural Data

No data identified

Other Relevant Studies

Steroid and xenobiotic receptor (SXR) is activated by endogenous and exogenous chemicals including steroids, bile acids, and prescription drugs. SXR is highly e xpressed in the liver and intestine, where it regulates cytochrome P450 3A4 (CYP3A4), which in turn controls xenobiotic and endogenous steroid hormone metabolism. Acetyl tributyl citrate (ATBC), an industrial plasticizer widely used in products such as food wrap, vinyl toys, and pharmaceutical excipients, strongly activated human and rat SXR. ATBC increased CYP3A4 messenger RNA (mRNA) levels and enzyme activity in the human intestinal cells but not in human liver cells. Similarly, CYP3A1 mRNA levels were in creased in the intestine but not the liver of ATBC-treated rats. These in vitro and in vivo results suggest that ATBC specifically induces CYP3A in the intestine by activating SXR. We suggest that ATBC-containing products be used cautiously because they ma y alter metabolism of endogenous steroid hormones and prescription drugs. (Takeshita A, 2011)

The octanol/water partition coefficient (log Kow) of ATBC was determined using HPLC to be 4.92 at 22°C and the EPIWIN KOWWIN model-predicted value (U.S. EPA, 2000c) was 4.29 at 25°C (EPA 2003).

In Vitro Toxicity Status

Carcinogenicity and Mutagenicity

Summary of In vitro and In vivo Mutagenicity/Genotoxicity Studies for ATBC

Test system	Test Object	Concentration of Substance	Results
Ames assay (preincubation method)	S. typhimurium TA98, TA100, TA1535, TA1537	50 to 5000 µg/plate	Negative a,b
Ames assay	S. typhimurium TA98, TA100,TA1535, TA1537, TA1538	333 to 10,000 µg/plate	Negative a,b
Ames assay	S. typhimurium TA98, TA100, TA1535, TA1537, TA1538	9 to 495 µg/plate	Negative b
In vitro chromosomal aberration assay	Rat lymphocyte cells	4 to 400 µg/ml	Negativea,b
In vivo/in vitro unscheduled DNA synthesis	Male rat primary cultures of hepatocytes	Single oral doses of 800 or 2000 mg/kg	Negative
Forward mutation assay	L5178Y (TK+/TK-) mouse lymphoma cells	200 to 480 µg/mla 10 to 230 µg/mlb	Negative a,b
Forward mutation assay	CHO/HGPRT	25 to 400 µg/ml	Negative a,b

a) with metabolic activation, b) without metabolic activation (EPA 2003)

The mutagenicity of acetyl tributyl citrate was evaluated using the Ames test in the *Salmonella typhimurium* strains: TA98, TA100, TA1535, TA1537, and TA1538. Acetyl tributyl citrate (in DMSO) was tested at 9, 50, 99, and 495 µM ± metabolic activation. Acetyl tributyl citrate was not mutagenic in any of the strains tested with or without metabolic activation (Johnson, 2002).

The mutagenicity of acetyl tributyl citrate was evaluated using the L5178Y (TK+/TK-) mouse lymphoma suspension/plate assay. Acetyl tributyl citrate (in DMSO) was tested at concentrations of 10 to 230 µg/mL (without metabolic activation) and 200 to 480 µg/mL (with metabolic activation). Acetyl tributyl citrate was not mutagenic with or without activation (Johnson, 2002).

Other relevant studies

The cytotoxicity of acetyl tributyl citrate and dibutyl sebacate was studied in cultured mammalian cells. Human oral epidermoid carcinoma (KB) cells, African green monkey kidney (Vero) cells, and Madin-Darby canine kidney (MDCK) cells were incubated with acetyl tributyl citrate or dibutyl sebacate over a range of concentrations for 72 hours. Cytotoxicity was evaluated by determining the extent of growth inhibition. The inhibited growth by 50 % of acetyl tributyl citrate in the various types were: 44.7 µg/mL in KB cells; 39.9 µg/m: in Vero cells; and 42.1 µg/mL in MDCK cells. The inhibited growth by 50 % of dibutyl sebacate in these cells were: KB cells, 1,549 µg/ml; Vero cells, 1,510 µg/ml; and MDCK cells, 1,549 µg/mL. In human KB cells, acetyl tributyl citrate is more toxic than tricresyl phosphate (TCP) and triphenyl phosphate (TPP). Acetyl tributyl citrate is less toxic than butylated hydroxyanisole (BHA), but shows toxicity similar to that of butylated hydroxytoluene (BHT) (Mochida, 1996).

Acetyl tributyl citrate was evaluated for its ability to induce the cytochrome P450-mediated fatty acid omega-hydroxylation system in rat liver microsomes, one of the initial cellular responses when animals are treated with peroxisome proliferators. The administration of acetyl tributyl citrate to rats increased the specific activity of laurate 12-hydroxylase from 2.8 (control) to 4.4 nmol 12-hydroxylaurate formed/min/nmol P450, respectively. Two protein bands, at 51 and 52 kDa, were found to increase 2-fold in acetyl tributyl citrate- treated rats (Okita, 1992).

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