BUTYRIC ACID

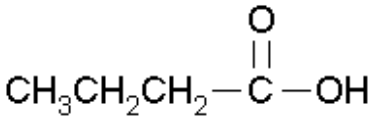
SYNONYMS

Butanoic acid
Butanic acid
1-Butyric acid
Ethylacetic acid
Propylformic acid
1-Propanecarboxylic acid

CHEMICAL FORMULA

$C_4H_8O_2$

CHEMICAL STRUCTURE



IDENTIFIER DETAILS

CAS Number	:	107-92-6
CoE Number	:	5
FEMA	:	2221
EINECS Number	:	203-532-3
E Number	:	-

CLP CLASSIFICATION

Ingredient CLP Classification: Yes

Endpoint	Classification	Category
Acute Oral Toxicity	-	-
Acute Dermal Toxicity	-	-
Acute Inhalation Toxicity	-	-
Skin Corrosive/irritant	Skin corrosion H314	1B
Eye Damage/Irritation	-	-
Respiratory Sensitisation	-	-
Skin Sensitisation	-	-
Mutagenicity/Genotoxicity	-	-
Carcinogenicity	-	-
Reproductive Toxicity		-
Specific Target Organ Toxicity	-	-
Aspiration Toxicity	-	-

REACH Statement

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

SPECIFICATIONS

Melting Point: -5.5°C [Sigma-Aldrich, 2002]
Boiling point: 163.5°C [Sigma-Aldrich, 2002]
Smiles code: C(C(O)=O)CC

STATUS IN FOOD AND DRUG LAWS

CoE limits:

Beverages (mg/kg)	Food (mg/kg)	Exceptions (mg/kg)
20	100	400 [candy and confectionery]

Acceptable Daily Intake:

ADI (mg/kg)	ADI Set by	Date Set	Comments
ACCEPTABLE	JECFA	1997	No safety concern at current levels of intake when used as a flavouring agent

FDA Status:[CFR21]

Section Number	Comments
182.60	Synthetic flavouring substances and adjuvants

HUMAN EXPOSURE

Natural Occurrence: Butyric acid normally occurs in butter as a glyceride; it had been reported found in the essential oils of: citronella Ceylon, *Eucalyptus globules, Araucaria cunninhamii, Lippia scaberrima, Monarda fistulosa,* cajeput, *Heracleum giganteum,* lavender, *Hedeoma pulegioides*, valerian, nutmeg, hops, *Pastinaca sativa*, Spanish anise, and others; it has been identified in strawberry aroma. Fenaroli, 2010].

The main natural occurrence in food [mg/kg]: apple juice: 2.5-5.4; other fruits: up to 5; bread: 7.6; fish: 3; Swiss cheese: 0-4, 290; other milk products: up to 115; beer: 0.6-2.6; other food and beverages: up to 75 [CoE, 1992].

Reported Uses: Butyric acid is reportedly used in baked goods at 105.3 ppm, fats and oils at 25.54 ppm, milk products at 15 ppm, frozen dairy at 13.76 ppm, meat products at 6.85 ppm, condiment and relishes at 200 ppm, soft candy at 41.62 ppm, sweet sauce at 8.56 ppm, gelatins and puddings at

21.15 ppm, snack foods at 48 ppm, non-alcoholic beverages at 16.49 ppm, alcoholic beverages at 3.54 ppm, imitation dairy at 183.1 ppm, and chewing gum at 10.81 ppm [Fenaroli, 2010].

TOXICITY DATA

Carmines (2002), Rustemeier *et al*., (2002), Roemer *et al*., (2002) and Vanscheeuwijck *et al.,* (2002) reported on a testing program designed to evaluate the potential effects of 333 ingredients added to typical commercial blended test cigarettes on selected biological and chemical endpoints. The studies performed included a bacterial mutagenicity screen [Ames assay] a mammalian cell cytotoxicity assay [neutral red uptake], determination of smoke chemical constituents and a 90-day rat inhalation study. Based on the findings of these studies, the authors concluded that the addition of the combined ingredients, including butyric acid at levels up to 3 ppm, “did not increase the overall toxicity of cigarette smoke” [Carmines, 2002].

Renne *et* al., (2006) evaluated the effects of tobacco flavouring and casing ingredients on both mutagenicity, and a number of physiological parameters in Sprague-Dawley (SD) rats. Test cigarettes containing a mixture of either 165 low-uses or eight high-use flavouring ingredients which included butyric acid at 1.3 ppm, were compared to a typical commercial tobacco blend without flavouring ingredients. The Ames assay (TA 98, 100,102, 1535 and 1537 ±S9) did not show any increase in Mutagenicity from “low” or “high” cigarette smoke condensate compared to the control. SD rats were exposed by nose-only inhalation for 1h/day, 5 days/wk for 13 weeks to smoke at concentrations of 0.06, 0.2 or 0.8mg/L from the test or reference cigarettes, or to air only. Plasma nicotine, COHb and respiratory parameters were measured periodically. Rats were necropsied after 13wk of exposure or following 13 wk of recovery from smoke exposure. Biological endpoints assessed included; clinical appearance, body weight, organ weights, and lesions (both gross and microscopic). The results of these studies did not indicate any consistent differences in toxicological effects between smoke from cigarettes containing the flavouring or casing ingredients and reference cigarettes.

In Vivo Toxicity Status

Species	Test Type	Route	Reported Dosage
Rat	LD_{50}	Oral	2940-8790mg/kg [JECFA, 1998]
Rat	LD_{50}	Oral	2000mg/kg
Rat	LD_{LO}	Oral	1400mg/kg
Rat	LC_{LO}	Inhalation	> 500mg/m3
Mouse	LD_{LO}	Oral	33600mg/kg
Mouse	LC_{LO}	Inhalation	> 500mg/m3
Mouse	LD_{50}	Intraperitoneal	3180mg/kg
Mouse	LD_{50}	Subcutaneous	3180mg/kg

Mouse	LD_{50}	Intravenous	800mg/kg
Rabbit	LD_{50}	Dermal	530ul/kg
Hamster	LD_{LO}	Oral	14000mg/kg
Rabbit	Irritation	Dermal	500mg
Rabbit	Irritation	Dermal	20mg/25 hours

[RTECS, 2002]

JECFA examined the toxicity of butyric acid, and estimated the daily per capita intake of 10000 μg/day, equivalent to 170 μg/kg bw/day, for Europeans. They concluded that there was no safety concerns based on the current levels of intake from food [JECFA, 1998].

In a study designed to look at the development of gastric lesions in rats with diets containing fatty acids, rats were fed a rice diet containing 1% butyric acid which was equivalent to 500 mg/kg/day. The concentration was gradually increased to 10% of the diet [5000 mg/kg/day] over a period of 500 days and had fore stomach lesions with prominent keratin cysts after being fed the diet for more than 50 days. No lesions were reported in the glandular stomach. The no observed effect level [NOEL] was reported to be 500 mg/kg/day [JECFA, 1998].

Carcinogenicity and Mutagenicity

A mouse skin painting study [Gaworski *et al.*, 1999], investigated the carcinogenicity of condensate prepared from cigarettes containing a number of additives in combination, including butyric acid at 2 ppm. The authors concluded that the study "did not indicate any substantive effect of these ingredients on the tumorigenicity of cigarette smoke condensate". It should be noted that the cigarettes contained a typical American blend humectant and sugar component [*i.e.* glycerine ≈ 20,000 ppm, propylene glycol at ≈ 24,000 ppm, and brown invert sugar at ≈ 24,000 ppm] [Gaworski *et al.*, 1999].

In an A/J mouse strain inoculated subcutaneously with C1300 murine neuroblastoma subcutaneously, the intraperitoneal administration of butyric acid was reported to be ineffective in preventing the growth of implanted tumours once they had reached 1-1.5 cm in diameter [Opdyke, 1981].

Dermal Toxicity

Butyric acid has been reported to be a moderately strong irritant of guinea pig skin. When applied at full strength to rabbit skin for 24 hours to either, abraded or intact skin under an occluded patch, it caused moderate to severe irritation. In a human volunteer study, when applied at 1 % in petrolatum, butyric acid was not an irritant when applied for 48 hours via an occluded patch. When tested in 25 human volunteers following a maximisation procedure, butyric acid when applied at 1 % in petrolatum was not found to be a sensitizer of human skin [Opdyke, 1981].

Inhalation Toxicity

Rats exposed to butyric acid vapour at 5.1-5.5 mg/l suffered only slight eye and/or nasal irritation during exposure, and for several hours after removal from the chamber [Hoffman *et al.*, 1991]. Continuous inhalation of up to 200 mg/m^3, by rats, for seven months produced a slight pulmonary reaction, but no other histological changes, with the suggested maximum exposure being recommended to be 100 mg/m^3 [Leung *et al.,* 1990]. An eight hour inhalation of vapour saturated with butyric acid caused no deaths in exposed rats (no further details were given) [Opdyke, 1981].

Prolonged exposure for an unspecified duration of rats, mice and rabbits to an atmospheric concentration of 0.1 - 0.2 mg/L reported to cause a massive increase in circulating neutrophils, lymphocytes which was attributed to the irritant nature of butyric acid. Rabbits exposed to butyric acid aerosol (40 mg/ml) were reported to have increased lethargy and dyspnea. Pathological investigation provided evidence of bronchiole and capillary dilation with emphysema [HSDB, 2003].
When tested at 3 ppm in cigarettes, in a 13 week inhalation study, the presence of butyric acid "…had no discernible effect on the character of extent of the biologic responses normally associated with inhalation of mainstream cigarette smoke in rats."[Gaworski *et al.*, 1998]. However, it should be noted that the cigarettes had been spiked with a number of flavour ingredients in combination prior to smoking, and they contained a typical American blend humectant and sugar component [*i.e.* glycerine ≈ 20,000 ppm, propylene glycol at ≈ 24,000 ppm, and brown invert sugar at ≈ 24,000 ppm].

A study investigated the effect of cigarettes containing various additives in three combinations, in a 90 day nose-only smoke inhalation study in rats. These ingredients included butyric acid at 3 ppm, a level described as a multiple of its typical use in a US cigarette. The data from this study, along with that from a number of other biological and chemical studies, indicate that the addition of the combined ingredients "did not increase the inhalation toxicity of the smoke, even at the exaggerated levels used" [Vanscheeuwijck *et al.*, 2002].

The addition of butyric acid at 129 ppm to reference cigarettes, used in a 90 day-sub-chronic inhalation exposure in rats, led to a series of pathological changes to smoke exposure that were indistinguishable from those changes caused by the control cigarettes. This indicated that addition of butyric acid to a reference cigarette had no discernable effect upon the type or severity of the treatment related pathological changes associated with tobacco smoke exposure [Baker *et al.,* 2004].

A re-analysis of a recent Health Hazard Evaluation (HHE) that was performed by the US National Institute for Occupational Safety and Health (NIOSH) was presented regarding the pulmonary status of workers at a flavorings manufacturing facility. The facility had used acetaldehyde, acetoin, benzaldehyde, butyric acid, diacetyl and many other flavouring chemicals for many years. Ten years of spirometry testing and job descriptions data on 112 workers were analysed by the authors and by NIOSH. Using NIOSH's

exposure assessment criteria, the prevalence of restrictive findings (as determined by spirometry testing) was compared in production workers to an internal control group that had reduced or no potential for exposure to flavouring chemicals. NIOSH used multiple linear regression to evaluate changes in pulmonary function by the exposure group. After a review of the NIOSH findings, associations between longitudinal changes in pulmonary health and workplace exposures through the use of generalized estimating equations were evaluated. The results were then compared to those obtained by NIOSH. The prevalence of pulmonary restriction was similar in production workers and internal controls. There was no relationship between the magnitude of exposure to flavourings chemicals and observed decrements in pulmonary function. The findings were contrary to those reported by NIOSH, which the authors suggest is most likely because of how the longitudinal nature of the spirometric data was accounted for. The authors concluded that many years of exposures to flavouring chemicals in this facility, including diacetyl, were not found to produce an increased risk of abnormal spirometric findings [Ronk *et al*., 2013].

Roemer (2014) and Schramke (2014) reported on a testing program designed to evaluate the potential effects of 350 ingredients added to an experimental kretek cigarette on selected biological and chemical endpoints. The studies performed included a bacterial mutagenicity screen [Ames assay] a mammalian cell cytotoxicity assay [neutral red uptake], Mouse Lymphoma assay, determination of smoke chemical constituents, a 4-day in vivo micronucleus assay and a 90-day rat inhalation study. Based on the results of these studies, the authors concluded that the addition of ingredients commonly used in the manufacture of kretek cigarettes, including Butyric acid at levels up to 156 ppm, did not change the overall in vivo/vitro toxicity profile of the mainstream smoke.

Other relevant studies

The absorption of butyric acid has been reported to be passive in the buccal cavity with migration into the lipid membrane of the mouth [Opdyke, 1981]. The production of butyric acid by bacterial fermentation of dietary carbohydrates has been noted in both ruminants and non-ruminants being readily absorbed through the epithelial lining of the rumen, caecum and colon. It is primarily absorbed from the small intestine in the non-esterified form via the portal route in directly to the liver. In the presence of the enzyme octanoyl coenzyme A synthetase, butyric acid is reportedly oxidised to butyryl coenzyme A ester, the major proportion of this compound is then catabalised to acetyl coenzyme A with the remaining butyric acid undergoing ω-oxidation to form succinic acid [Opdyke, 1981]. Butyric acid was reported to be metabolised largely to acetic acid in rats. Butyric acid metabolism in the rat also gives rise to the formation of ketone bodies, acetone, acetic acid, beta-hydroxybutyrate, acetoacetate that may then undergo fatty acid metabolism or are excreted in the urine [HSDB, 2003].

Butyric acid has been demonstrated to affect the gastric emptying rate via the interaction with duodenal receptors when administered intragastrically to

human subjects [concentration not specified]. After intravenous injection in to the abomasums of both cows and goats, the emptying time of the abomasums was reported to be increased [Opdyke, 1981].

Other than sensitisation reactions, organic acids are reported to have rarely induced any forms of chronic toxicity. Unlike highly reactive molecular structures such as epoxide or nitroso groups the carboxylic groups does not interact with cellular macromolecules or DNA. Organic acids are therefore considered to have a low carcinogenic potential and hence few have been tested in carcinogenicity studies [Leung *et al.,* 1990].

Butyric acid when administered intravenously at a concentration of $\leq$ 0.1 %, was reported to accelerate the jejunal villi movement in anaesthetised dogs with concentrations in excess of 0.1% causing a decrease in villi movement [Opdyke, 1981].

Intravenous infusions of butyrate to 4-6 month old lambs produced a small increase in plasma pancreatic glucagons and caused a large increase in mean plasma insulin concentration [HSDB, 2003].

The effect of feeding butyric acid on disaccharidase activity in the intestine and kidney was tested in control and chemically induced diabetic rats were studied. The experimental groups received 0, 250, 500 and 750 mg/kg/day. The increased activities of intestinal maltase, sucrase and lactase were significant reduced in the fibre fed diabetic group, with supplemental feeding of 500 mg/kg of butyric acid showed a further decrease in activities. In the kidney, the activity of disaccharidase was decreased in diabetic rats and significantly improved in the fibre fed group, being further improved in rats fed 500 mg/kg/day of butyric acid [Chethankumar *et al.,* 2002].

Behavioural Data

No data identified

***In Vitro* Toxicity Status**

Carcinogenicity and Mutagenicity

Butyric acid was negative in the Ames *Salmonella typhimurium* assay when tested at concentrations up to 10 mg/plate in strains TA92, TA94, TA98, TA100, TA1535 and TA1537, both with and without metabolic activation. Similarly, butyric acid was negative in a chromosomal aberration assay using the Chinese hamster lung fibroblast cell line at concentrations up to 1 mg/ml, both with and without metabolic activation [Ishidate *et al.*, 1984].

Butyric acid has also been demonstrated to be negative in the Ames assay with *Salmonella typhimurium* strains TA97 and TA102, at concentrations between 0.1-10 mg/plate [Fujita *et al.,* 1992].

Roemer *et al.,* (2002) reported on a study in which cigarettes containing various additives in three different combinations were produced. Smoke condensates prepared from these cigarettes were then tested in two different *in vitro* assays. The mutagenicity of the smoke condensate was assayed in the *Salmonella* plate incorporation [Ames] assay with tester strains TA98, TA100, TA102, TA1535 and TA1537 in the presence and absence of an S9 metabolic activation system. The cytotoxicity of the gas/vapour phase and the particulate phase was determined in the neutral red uptake assay with mouse embryo BALB/c 3T3 cells. The authors concluded that the *in vitro* mutagenicity and cytotoxicity of the cigarette smoke was not increased by the addition of the ingredients which included butyric acid at levels up to 3 ppm [a multiple of its typical use in a US cigarette] [Roemer *et al.,* 2002].

Baker *et al.,* [2004]; examined the effects of the addition of 482 tobacco ingredients upon the biological activity and chemistry of mainstream smoke. The ingredients, essentially different groups of flavourings and casings, were added in different combinations to reference cigarettes. The addition of butyric acid at 129 ppm was determined not to have affected the mutagenicity of the total particulate matter (TPM) of the smoke in either the Ames, *in vitro* micronucleus assay or the neutral red assay when compared with that of the control cigarettes [Baker *et al.,* 2004].

The mutagenicity of the smoke condensate was assayed in the *Salmonella* plate incorporation [Ames] assay with the tester strain TA98 in the presence of an S9 metabolic activation system. The cytotoxicity of the cigarette condensate was determined in the neutral red uptake assay and the (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H tetrazolium, inner salt assay (MTS assay) with the human hepatocellular liver carcinoma cell line, HEP-G2. It was concluded that the *in vitro* mutagenicity and cytotoxicity of the cigarette smoke was not increased by the addition of the ingredients, which included butyric acid at levels up to 127 ppm.

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

Roemer (2014) and Schramke (2014) reported on a testing program designed to evaluate the potential effects of 350 ingredients added to an experimental kretek cigarette on selected biological and chemical endpoints. The studies performed included a bacterial mutagenicity screen [Ames assay] a mammalian cell cytotoxicity assay [neutral red uptake], Mouse Lymphoma assay, determination of smoke chemical constituents, a 4-day in vivo micronucleus assay and a 90-day rat inhalation study. Based on the results of these studies, the authors concluded that the addition of ingredients commonly used in the manufacture of kretek cigarettes, including Butyric acid at levels up to 156 ppm, did not change the overall in vivo/vitro toxicity profile of the mainstream smoke.

Other relevant studies

A review of the anti-carcinogenic potential of butyric acid suggests that it may act by a number of mechanisms. It is a potent inhibitor of cellular proliferation, an inducer of differentiation and apoptosis in a number of cancer cell lines. It is associated with down regulation, or inactivation, of oncogene expression via the mechanisms of DNA hypermethylation or histone hyperacetylation. It may also play a role in the prevention of tumour invasiveness and metastasis by inhibiting urokinase, a facilitator of malignant cell penetration of the substratum. The increased production of colonic butyrate is considered to be one factor associated with the beneficial effects of increased fibre consumption [Rottleb *et al.,* 1996; Parodi, 1997].

The treatment of human HuH-7 hepatocellular carcinoma cells with sodium butyrate at concentrations of 3mM or higher, caused growth inhibition and led to nuclear fragmentation and DNA ladder formation, which is typical characteristic of apoptosis [Yamamoto *et al.,* 1998].

Butyric acid has been reported to induce differentiation of human leukaemia, including HL-60 cells, with reactive oxygen species being generated in butyric acid treated cells. The migratory and invasive properties of HL-60 cells were improved by butyric acid administration [Hollender *et al.,* 2002].

Butyrate is reported to stimulate the proliferation of normal crypt cells, where as it inhibits the growth and induces apoptosis of colon cancer cells [Zgouras *et al.,* 2003]. During the process of tumorigenesis most colon cancers are reported to acquire resistance to apoptosis. Butyric acid is well established as an antitumour agent that selectively targets cancer cells inducing apoptosis, but not in normal intestinal cells. Butyric acid was found to induce apoptosis in Caco-2 cells via the mitochondrial pathway [Ruemmele *et al.,* 2003].

Seto et al., (2008) examined the interaction between butyric acid and TNF-alpha in Jurkat T-cell apoptosis. Simultaneous treatment with TNF-alpha enhanced butyric acid-induced apoptosis by promoting caspase activity more than was achieved by either reagent alone. Expression of cFLIP (the cellular FLICE (FADD-like IL-1beta-converting enzyme)-inhibitory protein) decreased with increased concentrations of butyric acid and exogenous expression of cFLIP protein suppressed the enhancing effect by TNF-alpha in apoptosis. The authors concluded that butyric acid downregulated cFLIP expression and increases the susceptibility to TNF-alpha by activating caspases via the death receptor signal (Seto et al., 2008).

In a study conducted by Kurita-Ochiai *et al*., (2010) the authors aimed to determine the involvement of oxidative stress in apoptosis pathways, by examining the contribution of ROS in mitochondrial signaling pathways, death-receptor-initiated signaling pathway, and endoplasmic reticulum stress in butyric-acid-induced T-cell apoptosis. N-acetyl-L-Cysteine (NAC) abrogated mitochondrial injury, cytochrome c, AIF, and Smac release, and Bcl-2 and Bcl-xL suppression and Bax and Bad activation induced by butyric acid. However, the decrease in cFLIP expression by butyric acid was not restored by

treatment with NAC; increases in caspase-4 and -10 activities by butyric acid were completely abrogated by NAC. NAC also affected the elevation of GRP78 and CHOP/GADD153 expression by butyric acid. These results suggest that butyric acid is involved in mitochondrial-dysfunction- and endoplasmic reticulum stress-mediated apoptosis in human Jurkat T-cells via a ROS-dependent mechanism.

Short-chain fatty acids, such as butyric acid and propionic acid, are metabolic by-products generated by periodontal microflora such as Porphyromonas gingivalis, and contribute to the pathogenesis of periodontitis. However, the effects of butyrate on the biological activities of gingival fibroblasts (GFs) are not well elucidated. Human gingival fibroblasts (GFs) were exposed to various concentrations of butyrate (0.5 - 16 mm) for 24 h. Viable cells that excluded trypan blue were counted. Cell cycle distribution of GFs was analysed by propidium iodide-staining flow cytometry. Cellular reactive oxygen species (ROS) production was measured by flow cytometry using 2',7'-dichlorofluorescein (DCF). Total RNA and protein lysates were isolated and subjected to RT-PCR using specific primers or to western blotting using specific antibodies, respectively. Butyrate inhibited the growth of GFs, as indicated by a decrease in the number of viable cells. This event was associated with an induction of G0/G1 and G2/M cell cycle arrest by butyrate (4-16 mm) in GFs. However, no marked apoptosis of GFs was noted in this experimental condition. Butyrate (> 2 mm) inhibited the expression of cdc2, cdc25C and cyclinB1 mRNAs and reduced the levels of Cdc2, Cdc25C and cyclinB1 proteins in GFs, as determined using RT-PCR and western blotting, respectively. This toxic effect of butyrate was associated with the production of ROS (Chang *et al*., 2013).

PYROLYSIS AND TRANSFER STUDIES

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

REFERENCES

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

Carmines (2002). Evaluation of the potential effects of ingredients added to cigarettes. Part 1: Cigarette design, testing approach, and review of results. *Fd Chem Toxicol* **40**, 77-91.

Chang MC, Tsai YL, Chen YW, Chan CP, Huang CF, Lan WC, Lin CC, Lan WH, Jeng JH (2013). Butyrate induces reactive oxygen species production

and affects cell cycle progression in human gingival fibroblasts. *J Periodontal Res.* **48**(1):66-73.

Chethankumar *et al.,* (2002). Butyric acid modulates activities of intestinal and renal disaccharides in experimentally induced diabetic rats. *Nahrung* **46(5):** 345-348.

CoE (1992). Council of Europe. Flavouring substances and natural sources of flavourings. Vol 1 Chemically defined flavouring substances 4th Edition. Strasbourg.

Fenaroli (2010). Fenaroli's Handbook of Flavor Ingredients, 6th Edition

Fujita *et al.,* (1992). Mutagenicity of test of food additives with Salmonella typhimurium TA97, TA102. *Ann Rep. Tokyo Metr Res Lab*. **43:** 219-227.

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

Gaworski *et al.*, (1999). Toxicologic evaluation of flavor ingredients added to cigarette tobacco: skin painting bioassay of cigarette smoke condensate in SENCAR mice. *Toxicology*, **139**, 1-17.

Hoffman *et al.* (1991). Acute inhalation studies of monocarboxylic acids in rats - propionic acid, butyric acid, heptanoic acid and pelargonic acid. *Toxicologist*, **11**, 146.

Hollender *et al.,* (2002). Butyric acid increases invasiveness of HL-60 cells: role of reactive oxygen species. *FEBS Letters* **518(1-3):** 159-163.

HSDB [Hazardous Substances Data Base] [2003]. Database searched on 21/07/02 [http://sis.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB].

In vitro toxicity testing of tobacco ingredients in burnt form (Internal document R-53).

Ishidate *et al.* (1984). Primary mutagenicity screening of food additives currently used in Japan. *Fd. Chem. Toxic.* **22**, 623.

ITL internal report titled: Report on the Thermochemical Properties of Ingredients.

JECFA, (1998). Safety evaluation of certain food additives and contaminants. Prepared by the 49th meeting of the Joint FAO/WHO Expert Committee on Food Additives.

Kurita-Ochiai T, Ochiai K. (2010). Butyric acid induces apoptosis via oxidative stess in Jurkat T-cells. J Dent Res. Jul;89(7):689-94.

Leung *et al.,* (1990). Organic acids and bases: review of toxicological studies. *Am. J. Ind. Med.*, **18**, 717.
Opdyke (1981). Monographs on Fragrance Raw Materials: Butyric acid. *Fd. Cosmet. Toxicol.* **19**, 97-99.

Parodi (1997). Cows milk fat components as potential anticarcinogenic agents. *J. Nutr.*, **127**, 1055.

Renne, R.A., Yoshimura, H., Yoshino, K., Lulham, G., Minamisawa, S., Tribukait. Dietz, D.D., Lee, K.M., Westerberg, R.B. (2006). Effects of flavouring and casing ingredients on the toxicity of mainstream cigarette smoke in rats. *Inhalation Toxicology*. **18**:685-706.

Roemer (2014) Toxicological assessment of kretek cigarettes: Part 1: background, assessment approach, and summary of findings. Regul Toxicol Pharmacol.; **70** Suppl 1: 2-14.

Roemer (2014) Toxicological assessment of kretek cigarettes Part 6: the impact of ingredients added to kretek cigarettes on smoke chemistry and in vitro toxicity. Regul Toxicol Pharmacol.; **70** Suppl 1: 66-80.

Roemer *et al.,* (2002). Evaluation of the potential effects of ingredients added to cigarettes. Part 3: In vitro genotoxicity and cytotoxicity. *Fd Chem Toxicol* **40**, 105-111.

Ronk CJ, Hollins DM, Jacobsen MJ, Galbraith DA, Paustenbach DJ (2013). Evaluation of pulmonary function within a cohort of flavorings workers. *Inhal Toxicol*. **25**(2):107-17.

Rottleb *et al.,* (1996). Structure activity relationships of 17 structural analogues of N-butyric acid upon c-myc expression. *Int J. Cancer* **67:** 724-729.

RTECS [Registry of Toxic Effects of Chemicals] Search carried out on 20/09/02. RTECS No. ES5425000.

Ruemmele *et al.,* (2003). Butyrate induced Caco-2 cell apoptosis is mediated via the mitochondrial pathway. *Gut.* **52(1):** 94-100.

Rustemeier *et al.,* (2002). Evaluation of the potential effects of ingredients added to cigarettes. Part 2: Chemical composition of mainstream smoke. *Fd Chem Toxicol* **40**, 93-104.

Schramke (2014) Toxicological assessment of kretek cigarettes. Part 7: the impact of ingredients added to kretek cigarettes on inhalation toxicity. Regul Toxicol Pharmacol; **70** Suppl 1: 81-9.

Seto S., Kurita-Ochiai T., Ochiai K (2008). Increased susceptibility to tumor necrosis factor-alpha in butyric acid-induced apoptosis is caused by

downregulation of cFLIP expression in Jurkat T cells. *Microbiol Immunol*. **52**(3):188-96.

Sigma-Aldridge (2002). Searched on 20/09/02. [http://www.sigmaaldridge.com/cgi-bin].

Vanscheeuwijck *et al.,* (2002). Evaluation of the potential effects of ingredients added to cigarettes. Part 4: Subchronic inhalation toxicity. *Fd Chem Toxicol* **40**, 113-131.

Yamamoto *et al.,* (1998). Suppression of the growth of hepatocellular carcinoma by sodium butyrate *in vitro* and *in vivo*. *Int J Cancer*. **76(6):** 897-902.

Zgouras *et al.,* (2003). Butyrate impairs intestinal tumour cell-induced angiogenesis by inhibiting HIF-1alpha nuclear translocation. *Biochem. Biophys. Res. Commun*. 300(4): 832-838.