

**Evaluation of
Butyric acid
for Use as a Cigarette Ingredient**

November 2005

INTRODUCTION

Butyric acid (CAS # 107-92-6) is currently used worldwide at levels below **5 ppm** in selected cigarette brands manufactured and/or distributed by Philip Morris International. This document is a review of current published toxicology information on butyric acid abstracted from online toxicity databases.

TOXICITY DATA ON UN-BURNED MATERIAL

The following information was generated from the MICROMEDEX database tool <http://csi.micromedex.com> on November 28th 2005, unless otherwise indicated.

Overview

The following information was generated from the HSDB – Hazardous Substances Data Bank, a database of MICROMEDEX Systems (<http://csi.micromedex.com>) on November 28th 2005.

Butyric acid is a carboxylic acid known for its unpleasant rancid odour. It is commonly used in plastics, as a leather tanning agent. It is also used in foods as a flavourant such as butter, cheese, caramel, etc.

As a food flavouring additive, the material has been assessed under the provisions of the *Federal Food, Drug and Cosmetic Act, section 201 (s)*, by the Expert Committee of the USA Flavour and Extract manufacturer's Association (FEMA), to be generally recognized as safe (GRAS) under current conditions of use.

The Joint FAO/WHO Expert Committee on Food Additives has assessed butyric acid as presenting no safety concerns at current levels of intake when used as a flavouring agent. The daily per capita intake is estimated at 98 µg/kg bw/day in the USA and 170 µg/kg bw/day in Europe¹. It has also been defined as a flavouring substance which may be used as foodstuffs by the *Council of Europe* Committee of Experts on Flavouring Substances at an upper level of 100 mg/kg in foods.

Butyric acid is a common cosmetic ingredient.

This material appears on the list of "Permitted Additives to Tobacco Products in the United Kingdom" (Department of Health, 2003) at a maximum level permitted for inclusion in cigarettes of 0.15 % w/w tobacco.

In a study of the development of gastric lesions with diets containing fatty acids, rats fed a rice diet with 1% butyric acid (equivalent to 500 mg/kg bw per day) that was gradually increase to 10% (equivalent to 5000 mg/kg bw per day) over a period of 500 days had forestomach lesions with prominent keratin cysts after being fed the diet for more than 50 days. No lesions were observed in the glandular stomach¹.

¹ Safety evaluations of certain food additives and contaminants, WHO Food Additive Series 40: Saturated aliphatic acyclic linear primary alcohols, aldehydes, and acids.
<http://www.inchem.org/documents/jecfa/jecmono/v040je10.htm>

When butyric acid, valeric acid and octanoic acid were given daily by tracheal intubation on days 6 to 15 of gestation, only fetotoxicity was reported at the highest dose level (1500 mg/kg bw per day) with octanoic acid; no other evidence of fetotoxicity, developmental toxicity or teratogenicity associated with these three carboxylic acids was observed¹.

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Non-Human Toxicity Excerpts

1. Butyric acid rated 9 on rabbit eyes. /... Tested externally on eyes ... & have been rated numerically on a scale of 1-10 according to degree of injury observed after 24 hr, paying particular attention to condition of cornea. Most severe injuries have been rated 10./ **[**PEER REVIEWED**]** [Grant, W. M. Toxicology of the Eye. 2nd ed. Springfield, Illinois: Charles C. Thomas, 1974., p. 1139]
2. ... It is a moderately strong irritant in the guinea pig. In the closed, rabbit skin patch irritation test, 10 mg butyric acid produced a severe reaction in 24 hr, but in the open test, 500 mg elicited only a moderate response. **[**PEER REVIEWED**]** [Clayton, G. D. and F. E. Clayton (eds.). Patty's Industrial Hygiene and Toxicology: Volume 2A, 2B, 2C: Toxicology. 3rd ed. New York: John Wiley Sons, 1981-1982., p. 4914]
3. Prolonged exposure (of unspecified duration) of mice, rats, and rabbits to an atmospheric concentration of 0.1 to 0.2 mg/l butyric acid caused a massive increase in circulating lymphocytes and neutrophils, attributed to the irritant nature of the compd. After a 90 min exposure to a butyric acid aerosol (40 mg/l), rabbits displayed increased lethargy and dyspnea. Pathologic examination showed signs of bronchial and capillary dilation and emphysema. No lethalties /reported/ when rats were exposed for 8 hr to air saturated with butyric acid vapor. **[**PEER REVIEWED**]** [Clayton, G. D. and F. E. Clayton (eds.). Patty's Industrial Hygiene and Toxicology: Volume 2A, 2B, 2C: Toxicology. 3rd ed. New York: John Wiley Sons, 1981-1982., p. 4914]
4. A cell line derived from hamster spleen transformed by human papovavirus BK was adapted to grow in 5 mM butyrate. Ultrastructurally, the butyrate-adapted cells were found to contain large numbers of type-R and intracytoplasmic type-A virus-like particles. The unadapted cells contained only rare virus-like particles. **[**PEER REVIEWED**]** [Tralka TS et al; Proc Soc Exp Biol Med 174 (1): 41-6 (1983)]
5. In cattle following a single rapid iv injection of a sodium anion solution, butyrate was toxic at doses above 1 mmole/kg and progressively affected both the rate and progress of bromosulphthalein clearance as the dose increased. The butyrate effects were accentuated when the anion soln was injected at low pH where a large portion of the /compound/ would be unionized. Levels of butyrate in the rumens of feedlot cattle were high enough to provide toxic doses of these anions. **[**PEER REVIEWED**]** [Bide BW; Can J Comp Med 47 (2): 222-29 (1983)]
6. The role of the autonomic innervation in the control of pancreatic endocrine responses to iv infusions of butyrate was investigated in conscious 4-6 month old weaned lambs. Iv butyrate produced a small rise in mean arterial plasma pancreatic glucagon concentration which was unlikely to have had any physiological effect and produced no consistent or statistically significant changes in mean plasma pancreatic polypeptide concentration in

- any of the groups studied. In contrast, butyrate produced an abrupt and substantial rise in mean plasma insulin concentration which rose to a peak incremental value of about 300 pica mol/l in normal control. [**PEER REVIEWED**] [Bloom SR, Edwards AV; J Physiol 364: 281-8 (1985)]
7. In ewes, continuous infusion of butyrate decreased ruminal epithelial cell proliferation and caused a thickening of the stratum corneum. Daily short-term infusions produced stimulation of ruminal epithelial cell proliferation. Histological examination revealed parakeratotic changes in the stratum corneum. [**PEER REVIEWED**] [Galfi P et al; Zentralbl Veterinaermed, Reihe A 33 (1): 47-52 (1986)]
 8. Butyric acid was added to the normal diet of 5 day old chickens at 15 mM/l; the stimulating effect on keratinization was not restricted to ruminal epithelium, but also occurred in the crop. [**PEER REVIEWED**] [Galfi P et al; Zentralbl Veterinaermed, Reihe A 32 (2): 146-50 (1985)]
 9. The effects of butyrate on cellular morphology features and polyamine levels were examined in vitro in glioma F-98 cells. Following exposure to butyrate, the glioma appeared more differentiated and had more characteristics of normal glial cells, such as processes extension, lower nucleocytoplasmic ratio, contact inhibition, and monolayer growth. Butyrate increased glioma intracellular putrescine and spermine levels and decreased spermidine level. [**PEER REVIEWED**] [Lin LI, Lin JK; Taiwan I Hsueh Hui Tsa Chih 84 (5): 536-45 (1985)]
 10. Treatment of B16-F10 melanoma cells with butyric acid inhibited cell growth and delayed tumor appearance in syngeneic mice. Butyric acid, did not increase melanin content and decreased tyrosinase activity. [**PEER REVIEWED**] [Nordenberg J et al; Exp Cell Res 162 (1): 77-85 (1986)]
 11. Butyrate induces the formation of additional 15% new RNA sequences, essentially in the class of abundant sequences and of sequences of intermediary abundance. This drug also decreases 5-10 times the frequency of the sequences of intermediary abundance. [**PEER REVIEWED**] [Raymondjean M et al; Biol Cell 54 (1): 39-47 (1985)]
 12. The most effective inducer was butyrate, which caused an essentially linear increase in alkaline phosphatase activity in the concentration range of 2 to 10 mM. Butyrate and related short-chain fatty acids also suppressed 9-1C cell growth. Butyrate appeared to be unique among the various fatty acids in causing an increase in cell protein. Butyrate caused 9-1C cells, which normally grow in a disorganized array with no apparent affinity for each other, to spread out and become organized into parallel tracts through the monolayer. [**PEER REVIEWED**] [Reese DH et al; Cancer Res 45 (5): 2308-13 (1985)]
 13. Treated murine lymphosarcoma cells were incubated with different amounts of the polyanion heparin, which is known to interact predominantly with chromatin-associated histones. Unlike isolated histone H1, histone H1 in the nuclei of butyrate-treated cells displayed an enhanced affinity for the binding to heparin as compared to histone H1 from control cells. [**PEER REVIEWED**] [Stros M, Siroky J; Gen Physiol Biophys 4 (4): 375-81 (1985)]
 14. To understand corneal wound healing, in which fibronectin plays an important role, the regulatory mechanism of fibronectin synthesis was studied in cultured rabbit corneal

blocks in situ. The amount of fibronectin was detected by ELISA. Butyric acid stimulated fibronectin synthesis in a dose-response fashion, and had a greater stimulatory effect than did any of its derivatives examined. This suggests that butyrate stimulates fibronectin synthesis specifically. Additivity of stimulation by butyrate and by 8-bromo-cAMP was observed at the saturated concentration of each, indicating an independent mechanism of action for these two compounds. [**PEER REVIEWED**] [Tanaka H, Nishida T; J Cell Physiol 123 (2): 191-6 (1985)]

15. Propionic or butyric acid was added at sublethal doses (0.1-2 mg/ml) to a growth medium supporting growth of *Aspergillus flavus* and subsequent aflatoxin production. A reduction in growth and aflatoxin production occurred when the acids were added at the time of inoculation. Addition of the acids to cultures at different times resulted in little effect on growth but production of aflatoxin after 12 days was reduced with earlier time of application for both propionic and butyric acid. When the acids were added to rough rice with a moisture content of 21% and inoculated with *A. flavus* fungal growth and aflatoxin production were reduced relative to non-inoculated controls. [**PEER REVIEWED**] [Ghosh J, Haggblom P; Int J Food Microbiol 2 (16): 323-30 (1985)]

The following information was generated from the RTECS – Registry of Toxic Effects of Chemical Substances, a database of MICROMEDEX Systems (<http://csi.micromedex.com>) on November 28th 2005.

Health hazard data

Acute toxicity

LDLO/LCLO - LOWEST PUBLISHED LETHAL DOSE/CONC

Mouse

LDLo - ROUTE: Oral; DOSE: 500 mg/kg [Toksikologiya Novykh Promyshlennykh Khimicheskikh Veshchestv. Toxicology of New Industrial Chemical Substances. For English translation, see TNICS*. (Izdatel'stvo Meditsina, Moscow, USSR) No.1- 1961- (4,19,1962)]

TOXIC EFFECTS:

Gastrointestinal - Necrotic changes

Kidney, Ureter, and Bladder - Other changes

Blood - Changes in spleen

LD50/LC50 - LETHAL DOSE/CONC 50% KILL

Rat

LD50 - ROUTE: Oral; DOSE: 2 gm/kg [Toxicometric Parameters of Industrial Toxic Chemicals Under Single Exposure, Izmerov, N.F., et al., Moscow, Centre of International Projects, GKNT, 1982 (-,30,1982)]

Mouse

LD50 - ROUTE: Intraperitoneal; DOSE: 3180 mg/kg [Journal of Pharmacy and Pharmacology. (Pharmaceutical Soc. of Great Britain, 1 Lambeth High St., London SE1 7JN, UK) V.1- 1949- (21,85,1969)]

LD50 - ROUTE: Intravenous; DOSE: 800 mg/kg [Acta Pharmacologica et Toxicologica. (Copenhagen, Denmark) V.1-59, 1945-86. For publisher information, see PHTOEH (18,141,1961)]

TOXIC EFFECTS:

Behavioral - Convulsions or effect on seizure threshold

LD50 - ROUTE: Subcutaneous; DOSE: 3180 mg/kg [Journal of Pharmacy and Pharmacology. (Pharmaceutical Soc. of Great Britain, 1 Lambeth High St., London SE1 7JN, UK) V.1- 1949- (21,85,1969)]

Rabbit

LD50 - ROUTE: Skin; DOSE: 530 uL/kg [Union Carbide Data Sheet. (Union Carbide Corp., 39 Old Ridgebury Rd., Danbury, CT 06817) (4/10/1968)]

OTHER LD/LC - OTHER LETHAL DOSE/CONC

Rat

LC - ROUTE: Inhalation; DOSE: >500 mg/m³ [Toksikologiya Novykh Promyshlennykh Khimicheskikh Veshchestv. Toxicology of New Industrial Chemical Substances. For English translation, see TNICS*. (Izdatel'stvo Meditsina, Moscow, USSR) No.1- 1961- (4,19,1962)]

TOXIC EFFECTS:

Lung, Thorax, or Respiration - Structural or functional change in trachea or bronchi

Mouse

LC - ROUTE: Inhalation; DOSE: >500 mg/m³ [Gigiena i Sanitariya. For English translation, see HYSAAV. (V/O Mezhdunarodnaya Kniga, 113095 Moscow, USSR) V.1- 1936- (23(5),31,1958)]

TOXIC EFFECTS:

Lung, Thorax, or Respiration - Structural or functional change in trachea or bronchi

Irritation

SKIN - STANDARD DRAIZE TEST

Rabbit

ROUTE: Skin; DOSE: 20 mg/24H; REACTION: Moderate ['Prehled Prumyslove Toxikologie; Organicke Latky,' Marhold, J., Prague, Czechoslovakia, Avicenum, 1986 (-,306,1986)]

SKIN - OPEN DRAIZE TEST

Rabbit

ROUTE: Skin; DOSE: 500 mg ; REACTION: Moderate [Union Carbide Data Sheet. (Union Carbide Corp., 39 Old Ridgebury Rd., Danbury, CT 06817) (4/10/1968)]

Chicken

CELL TYPE: fibroblast; DOSE: 2 mmol/L [Cell (Cambridge, Mass.). (MIT Press, 28 Carleton St., Cambridge, MA 02142) V.1- 1974- (12,855,1977)]

Other multiple dose toxicity data

Rat

TDL₀ - ROUTE: Oral; DOSE: 14 gm/kg/7D continuous [Food and Chemical Toxicology. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.20- 1982- (29,367,1991)]

TOXIC EFFECTS:

Gastrointestinal - Other changes

Mouse

TDL₀ - ROUTE: Oral; DOSE: 33600 mg/kg/7D continuous [Food and Chemical Toxicology. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.20- 1982- (29,367,1991)]

TOXIC EFFECTS:

Gastrointestinal - Other changes

Hamster

TDL₀ - ROUTE: Oral; DOSE: 14 gm/kg/7D continuous [Food and Chemical Toxicology. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.20- 1982- (29,367,1991)]

TOXIC EFFECTS:

Gastrointestinal - Other changes

TOXICITY DATA ON BURNT MATERIAL

Data on the toxicity of butyric acid as a cigarette ingredient has been evaluated in a series of studies. The results of these studies may be found in the following references:

E.L. Carmines, 2002, "Evaluation of the Potential Effects of Ingredients Added to Cigarettes. Part I: Cigarette Design, Testing Approach and Review of Results," Food and Chemical Toxicology, 40:77-91. **PEER REVIEWED**

K. Rustemeier et al, 2002, "Evaluation of the Potential Effects of Ingredients Added to Cigarettes Part II. Chemical Smoke Composition," Food and Chemical Toxicology, 40:93 - 104. **PEER REVIEWED**

Roemer et al, 2002, "Evaluation of the Potential Effects of Flavor Ingredients Added to Cigarettes. Part 3. In Vitro Genotoxicity and Cytotoxicity," Food and Chemical Toxicology, 40:105-111. **PEER REVIEWED**

P.M. Vanscheeuwijck et al, 2002, "Toxicological Evaluation of Cigarettes without and with the Addition of Flavor Ingredients to the Tobacco. Part 4. Subchronic Inhalation Toxicity," Food and Chemical Toxicology, 40:113-131. **PEER REVIEWED**

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

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

These studies indicate that the ingredients used in the production of cigarettes do not increase the overall toxicity of cigarette smoke.

DATA ON THE EFFECTS ON HUMAN HEALTH

The following information was generated from the HSDB – Hazardous Substances Data Bank, a database of MICROMEDEX Systems (<http://csi.micromedex.com>) on November 28th 2005.

Human Toxicity Excerpts

1. Butyric acid can act as a mild irritant in man. ... Application to intact human skin elicits a moderate burning only after 52 min and erythema is hardly noticeable. Slight epidermal scaling may follow within 24 hr. [**PEER REVIEWED**] [Clayton, G. D. and F. E. Clayton (eds.). Patty's Industrial Hygiene and Toxicology: Volume 2A, 2B, 2C: Toxicology. 3rd ed. New York: John Wiley Sons, 1981-1982., p. 4914]
2. Two subpopulations of human colon tumor cells (clones A and D) which differ in their intrinsic sensitivity to X-ray were grown for several passages in tissue culture medium containing the differentiation-inducing agent sodium butyrate (2 mM). [**PEER REVIEWED**] [Arundel CM; Radiat Res 104 (3): 443-48 (1985)]
3. Butyrate induced a marked reduction in the growth rate, colony forming efficiency in soft agar and de novo synthesis of DNA as well as remarkable morphological changes including cell enlargement, flattening, and a decreased number of nucleoli. Secretion of alpha-fetoprotein was reduced during culture with butyrate, while that of albumin was increased. [**PEER REVIEWED**] [Nakagawa T et al; Br J Cancer 51 (3): 357-63 (1985)]

The following information was generated from the RTECS – Registry of Toxic Effects of Chemical Substances, a database of MICROMEDEX Systems (<http://csi.micromedex.com>) on November 28th 2005.

Health hazard data

Genetic effects

DNA DAMAGE

Human

CELL TYPE: HeLa cell; DOSE: 3 mmol/L [Cell (Cambridge, Mass.). (MIT Press, 28 Carleton St., Cambridge, MA 02142) V.1- 1974- (12,855,1977)]

DNA INHIBITION

Human

CELL TYPE: lymphocyte; DOSE: 4 mmol/L [Hematological Oncology. (John Wiley & Sons Ltd., Baffins Lane, Chichester, W.Sussex, PO19 1UD, UK) V.1- 1983- (2,381,1984)]

CONCLUSION

Cigarette smoking causes lung cancer, heart disease, emphysema and other serious diseases in smokers. Smokers are far more likely to develop serious diseases, like lung cancer, than non-smokers. There is no "safe" cigarette. Government health warnings about smoking apply to all cigarettes, regardless of the ingredients added, including those containing only tobacco and paper.

While Philip Morris International has not conducted human studies on the health effects of ingredients used in cigarette manufacture, studies have been conducted using scientifically accepted in vitro and in vivo toxicity assays with various ingredient mixtures (see Toxicity Data on Burnt Material above). These studies show there is no meaningful difference in the composition or toxicity of smoke when the smoke from cigarettes with added ingredients is compared to the smoke from cigarettes without added ingredients. These findings are supported by similar studies from the published literature. It is our scientific judgment, based on the best available data, that butyric acid used in our cigarettes does not increase the overall toxicity of cigarette smoke.