Toxicological profile for

Ethyl butyrate

This ingredient has been assessed to determine potential human health effects for the consumer. It was considered not to increase the inherent toxicity of the product and thus is acceptable under conditions of intended use.

1. Name of substance and physico-chemical properties

1.1. IUPAC systematic name

Ethyl butanoate (PubChem)

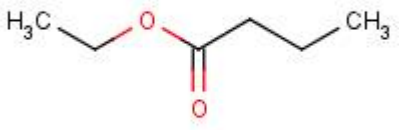
1.2. Synonyms

AI3-18427; BRN 0506331; Butanoic acid ethyl ester; Butyric acid ethyl ester; Butyric ester; Butyric ether; CCRIS 6554; EINECS 203-306-4; Ethyl butanoate; Ethyl butyrate; Ethyl butyrate (natural); Ethyl n-butyrate; FEMA No. 2427; HSDB 406; NSC 8857; UNII-UFD2LZ005D; 4-02-00-00787 (Beilstein Handbook Reference); UN1180 (ChemIDplus)

1.3. Molecular formula

C6H12O2 (ChemIDplus).

1.4. Structural Formula



1.5. Molecular weight (g/mol)

116.16 (ChemIDplus)

1.6. CAS registration number

105-54-4

1.7. Properties

1.7.1. Melting point

(°C): -98 (ChemIDplus; ChemSpider; EPISuite, 2017); -93 or -93.3 (ChemSpider); -97 (HSDB, 2015)

1.7.2. Boiling point

(°C): 121.5 (ChemIDplus; EPISuite, 2017); 119-121 (ChemSpider)

1.7.3. Solubility

4900 mg/L at 20°C (ChemIDplus; EPISuite, 2017); 6.2 g/L at 20°C (GSBL, 2021); 0.05 M [5808 mg/L] (PubChem); “Not miscible with water” (IGS, 2021)

1.7.4. pKa

No data available to us at this time.

1.7.5. Flashpoint

(°C): 26 or 19.4 (ChemSpider); 24 (closed cup) (NZ EPA CCID); 25 (closed cup) (IGS, 2021)

1.7.6. Flammability limits (vol/vol%)

“Upper explosion limit: 21.1 vol.% (200°C, 10 bar), lower explosion point: 18°C” (GESTIS);.

1.7.7. (Auto)ignition temperature

(°C): 463 (PubChem); 460 (IGS, 2021); 440 (GESTIS);

1.7.8. Decomposition temperature

(°C): No data available to us at this time.

1.7.9. Stability

Stable at room temperature in closed containers under normal storage and handling conditions; Stable, Flammable, Incompatible with strong oxidizing agents, acids, bases (ChemSpider).

1.7.10. Vapor pressure

12.8 mm Hg at 20°C ChemIDplus; EPISuite, 2017); 14 mm Hg at 20°C (HSDB, 2015); 16.5 mmHg (IGS, 2021); 17.5 hPa [13.1 mmHg] at 20°C (GESTIS)

1.7.11. log Kow

1.71 (GESTIS); 1.73 at 20°C (GSBL, 2021)

2. General information

2.1. Exposure

Occurs naturally in tobacco (Stedman, 1968)
Cosmetics: yes (Merck, 1996)
Food: yes (Burdock GA, 2010)
Environment: yes (HSDB, 2015)
Pharmaceuticals: yes (Martindale, 1993)

Reported levels from use as a flavouring (ppm): (FEMA, 1994).

Food category	Usual	Max.	Food category	Usual	Max.
Alcoholic beverages	16.05	23.97	Gelatins, puddings	56.37	82.15
Baked goods	95.87	136.60	Hard candy	59.74	168.00
Chewing gum	831.90	1393.00	Meat products	5.88	18.60
Fats, oils	15.67	25.01	Nonalcoholic beverages	25.23	37.88
Frozen dairy	39.68	66.63	Soft candy	71.52	104.10

Estimated intake from flavouring use: 0.2641 mg/kg bw/day.

As taken from Burdock, 2010.

Ethyl butyrate is used as a perfuming agent in cosmetics in the EU. As taken from CosIng.

Probable Routes of Human Exposure:

Workers that use or produce ethyl n-butyrate may breathe in mists or have direct skin contact. The general population may be exposed by breathing in mists or having direct skin contact when using perfumes containing ethyl n-butyrate or when eating foods containing this compound.

According to the 2012 TSCA Inventory Update Reporting data, 2 reporting facilities estimate the number of persons reasonably likely to be exposed during the manufacturing, processing, or use of ethyl n-butyrate in the United States may be as low as 50 workers and as high as 99 workers per plant; the data may be greatly underestimated due to confidential business information (CBI) or unknown values(1). [(1) US EPA; Chemical Data Reporting (CDR). Non-confidential 2012 Chemical Data Reporting information on chemical production and use in the United States. Available from, as of May 12, 2015: http://www.epa.gov/cdr/pubs/guidance/cdr_factsheets.html **PEER REVIEWED**

As taken from HSDB, 2015.

Ethyl butyrate is listed as a fragrance ingredient on the US EPA InertFinder Database (2022) and by IFRA.

Ethyl butyrate (CAS RN 105-54-4) is listed as an ingredient (at given concentrations, where specified) in auto, inside the home and personal care (at up to 1.0%) products by the CPID.

“Industrial Processes with risk of exposure: Painting (Solvents)”

As taken from Haz-Map, 2020

Ethyl butyrate (CAS RN 105-54-4) is used as a flavour enhancer in non-medicinal and general oral natural health products (Health Canada, 2021).

“Aims: To analyse content and emission data submitted by manufacturers for nicotine-containing vaping products in the United Kingdom (UK) in accordance with the European Union Tobacco Products Directive. Design: Analysis of ingredient and emission data reported for all e-liquid-containing e-cigarettes, cartridges or refill containers notified to the Medicines and Healthcare Regulatory Agency (MHRA) from November 2016 to October 2017. Setting: United Kingdom CASES: A total of 40 785 e-liquid containing products. Measurements: The average number of ingredients per product, nicotine concentrations, frequency of occurrence ingredients and frequency and levels of chemical emissions. Findings: Reports were not standardised in relation to units of measurement or constituent nomenclature. Products listed an average of 17 ingredients

and 3.3% were reported not to contain nicotine. A total of 59% of products contained <12 mg nicotine per mL, and <1% were reported to have nicotine concentrations above the legal limit of 20 mg/mL. Over 1500 ingredients were reported, and other than nicotine the most commonly reported non-flavour ingredients were propylene glycol (97% of products) and glycerol (71%). The most common flavour ingredients were ethyl butyrate (42%), vanillin (35%) and ethyl maltol (33%). The most frequently reported chemical emissions were nicotine (65%), formaldehyde (48%) and acetaldehyde (40%). The reporting of the concentration of emissions was not standardised; emissions were reported in a format allowing analysis of median estimated concentration for between 13% and 100% of products for each reported emission. Most of the frequently reported emissions, other than nicotine, were present in median estimated concentrations below 1 µg/L of inspired air, and with the exception of nicotine, acrolein and diacetyl, at median levels below European Chemicals Agency Long Term Exposure and United States (US) Department of Labor Occupational Safety and Health Administration (OSHA) limits, where these were available. Conclusions: An analysis of reports to the United Kingdom's Medicines and Healthcare products Regulatory Agency by manufacturers of vaping products shows that (i) these products have a large range of ingredients and emissions, (ii) the reporting system is unstandardized in terms of reporting requirements, and (iii) for quantified emissions, median levels are for the most part below published safe limits for ambient air." As taken from Nyakutsikwa B et al. 2021. Addiction. Epub ahead of print. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/33651418/

"Electronic cigarettes (e-cigarettes, EC) form an aerosol from the heating element and liquid-containing cartridge. The heating element aerosolizes the refill solutions (e-liquids) when the power source of e-cigarette is pressed. E-liquids consist of combinations of propylene glycol, glycerine, nicotine and flavouring ingredients. Puffing activates the battery-operated heating element in the atomizer and will produce smoke that is similar to conventional cigarette (CC). This study evaluated the chemical composition of e-liquid and aerosol samples available in Malaysia. We analyzed the volatile organic compounds in e-liquids and the aerosols samples from EC using gas chromatography mass spectrometer. Seventy-two EC e-liquids were analyzed through different flavours from more than 60 brands. Samples consisted of 32 nicotine-free (0 mg) and 40 nicotine-containing refill solutions (3 - 12 mg). Overall, 116 compounds were identified from EC e-liquids. On the other hand, 275 compounds were identified from their resultant aerosol samples. There were 42 compounds found in both e-liquids and aerosols. Seven compounds were only found in e-liquids and 38 compounds were only found in aerosols. Propylene glycol was found in all of the e-liquid and aerosol samples. Glycerin was found in 99% of the e-liquid and 100% of aerosol samples. At least 60% of the EC e-liquids and the resultant aerosol contain piperidine, butanoic acid ethyl ester and nicotine. It was also found that at least 9 out of 35 nicotine free labeled e-liquids contain nicotine. Some of these compounds were known to be detrimental to health and were detected in aerosol although they were not present in e-liquids. While some of the compounds are flavouring ingredients, it is necessary to evaluate its long-term effects on EC users." As taken from Nawi, MNM et al. 2020. Journal of Environmental Protection 11, 664-681. Available at https://www.scirp.org/pdf/jep_2020090915215877.pdf

2.2. Combustion products

This ingredient was investigated in a pyrolysis study. Results are given in JTI Study Report (s).

Compound	Two stage heating		One stage heating	
	Abundance	Area%	Abundance	Area%
ethyl butyrate	13240766839	99.94	14468427940	99.90

Total ion chromatogram	13248583503	100	14483055826	100

This ingredient was investigated in a pyrolysis study. Results are given in Baker and Bishop (2004) J. Anal. Appl. Pyrolysis, 71: 223-311.

Ingredient CAS Number	Chemical class	Mol. Wt. (M) bp or mp (°C)	Max. cig. Appln. Level (ppm)	Purity of sample pyrolysed	Composition of pyrolysate (Compound, %)	Max. level in smoke (µg)
Ethyl butyrate CAS 105-54-4	Ester	M=116 bp 121	250	98	Ethylbutyrate 100	125

In a pyrolysis study, 98% of ethyl butyrate added to cigarettes was transferred intact to the smoke (Purkis et al. 2011).

2.3. Ingredient(s) from which it originates

Natural Pollution Sources:

ETHYL BUTYRATE OCCURS NATURALLY IN BREWER'S YEAST. [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. 2284] **PEER REVIEWED**

Reported found in olive oil and other vegetable oils. Reported found in apple, banana, citrus peel oils and juices, cranberry, blueberry, black currants, guava, grapes, papaya, strawberry, onion, leek, cheeses, chicken beef, beer, cognac, rum, whiskies, cider, sherry, grape wines, coffee, honey, soybeans, olives, passion fruit, plums, mushrroms, mango, fruit brandies, kiwifruit, mussels and pawpaw. [Burdock, G.A. (ed.). Fenaroli's Handbook of Flavor Ingredients. 6th ed.Boca Raton, FL 2010, p. 575] **PEER REVIEWED**

Ethyl butyrate is a natural product of certain plants and has been detected in the volatile components from the following natural foods: U.S. blue cheese(1); Beaufort mountain cheese(2); dalieb fruit (Borassus aethiopum L.)(3); ripening bananas(4); commercial and concentrated aqueous orange essences(5); Concord grape essence(6); tree-ripened nectarines(7); and ripening kiwi fruit(8). [(1) Day EA, Anderson DF; J Agric Food Chem 13: 2-4 (1965) (2) Dumont JP, Adda J; J Agric Food Chem 26: 364-7 (1978) (3) Harper DB et al; J Sci Food Agric 37: 685-88 (1986) (4) Macku C, Jennings WG; J Agric Food Chem 35: 845-8 (1987) (5) Moshonas MG, Shaw PE; J Agric Food Chem 38: 2181-4 (1990) (6) Stevens KL et al; J Food Sci 30: 1006-7 (1965) (7) Takeoka GR et al; J Agric Food Chem 36: 553-60 (1988) (8) Bartley JP, Schwede,AM; J Agric Food Chem 37: 1023-5 (1989)] **PEER REVIEWED**

Food Survey Values:

Ethyl butyrate was detected, not quantified (detection limits listed if specified in source), in the following foods: U.S. blue cheese aroma fraction(1); Beaufort mountain cheese volatiles(2); volatile flavor components of dalieb fruit (Borassus aethiopum L.)(3); volatiles of ripening bananas (qualitatively detected 120 hr after unripened bananas were placed in glass test chamber; relative concn increased after initially detected through end of experiment - 10 days)(4); commercial and concentrated aqueous orange essences(5); Concord grape essence(6); tree-ripened nectarines(7). It was detected in the volatile components of ripening kiwi fruit at levels of 0.6% of the volatiles in mature fruit and 14.2% of the volatiles in ripe fruit(8). [(1) Day EA, Anderson DF; J Agric Food Chem 13: 2-4 (1965) (2) Dumont JP, Adda J; J Agric Food Chem 26: 364-7 (1978) (3) Harper DB et al; J Sci Food Agric 37: 685-88 (1986) (4) Macku C, Jennings WG; J Agric Food Chem 35: 845-8 (1987) (5) Moshonas MG, Shaw PE; J Agric Food Chem 38: 2181-4 (1990) (6) Stevens KL et al; J Food Sci 30: 1006-7 (1965) (7) Takeoka GR et al; J Agric Food Chem 36: 553-60 (1988) (8) Bartley JP, Schwede AM; J Agric Food Chem 37: 1023-5 (1989)] **PEER REVIEWED**

Ethyl n-butyrate was detected in the skin and pulp of Queen Anne's pocket melon (Cycumis melo, L. Cucurbitaceae) at 900 and 206.5 ug/kg equivalent of 2-octanol, respectively(1). It is present at 0.31 ug/kg fruit of Pineapple guava (Feihoa sellowiana Berg) and has been reported in volatiles of common guava (Psidium guajava , L.) and strawberry or yellow guava(Psidium cattleianum var)(2). Ethyl n-butyrate, present at 0.033 ppm, is a volatile flavor component of fresh grapefruit juice,(3). The compound was detected in Japanese muskmelon (var Miyabi) (Cucumis melo), contributing a grape-like odor(4). Ethyl n-butyrate concentrations in volatiles from fresh, hand-pressed, unpasteurized orange juice were (variety, ppm): Valencia, 0.84; Pineapple, 0.82; Hamlin, 0.70; navel, trace; Pera, 0.11; Ambersweet, 0.81(5). Ethyl n-butyrate was detected, not quantified in headspace volatiles of cabernet sauvignon wines from Napa Valley, CA(6). [(1) Aubert C, Pitrat M; J Agric Food Chem 54: 8177-82 (2006) (2) Binder RG, Flath RA; J Agric Food Chem 37: 734-6 (1989) (3) Cadwallader KR, Xu Y; J Agric Food Chem 42: 782-4 (1994) (4) Hayata Y et al; J Agric Food Chem 51: 3415-18 (2003) (5) Moshonas MG, Shaw PE; J Agric Food Chem 42: 1525-8 (1994) (6) Shimoda M et al; J Agric Food Chem 41: 1664-66 (1993)] **PEER REVIEWED**

Fish/Seafood Concentrations:

Ethyl butyrate was detected at a concn of 0.29 ppm in a sample of mussel (Mytilus edulis) collected on July 31, 1985 at the Oarai Coast in Ibaraki, Japan(1). It was not detected (detection limit not specified) in a sample of mussel collected at the same location on July 31, 1986(1). The compound was detected at in volatile components in salt-fermented pastes: 3740 ng/g anchovy (Engraulis japonica); 10,400 ng/g big-eyed herring (Harengula zunasi); and 74.9 ng/g hair-tail (Trichiurus japonica). It was not detected in shrimp paste (Acetes chinensis). Samples were obtained from a fish market in Masan, Korea(2). [(1) Yasuhara A, Morita M; Chemosphere 16: 2559-65 (1987) (2) Cha YJ, Cadwallader KR; J Food Sci 60: 19-24 (1995)] **PEER REVIEWED**] **PEER REVIEWED**

Plant concentrations:

Ethyl n-butyrate detections in plants(1).

Genus species	Family	Common name	Part	Concn (ppm)
Micromeria fruiticosa subsp barbata	Lamiaceae	Tea hyssop	Shoot	0-5
Citrus sinensis	Rutaceae	Orange	Fruit juice	1.02
Ananas comosus	Bromeliaceae	Pineapple	Fruit	not reported

Carica papaya	Caricaceae	Papaya	fruit	not reported
Malus domestica	Rosaceae	Apple	Plant	not reported
Passiflora edulis	Passifloraceae	Maracuya, Passionfruit	Fruit	not reported
Ribes nigrum	Grossulariaceae	Black current	Fruit	not reported
Asimina triloba	Annonaceae	Pawpaw	fruit	7.109

[(1) USDA; Dr. Duke's Phytochemical and Ethnobotanical Databases. Plants with a chosen chemical. Ethyl butyrate. Ethyl-Butanoate. Ethyl Butyrate. Washington, DC: US Dept Agric, Agric Res Service. Available from, as of Jun 5, 2105: http://www.ars-grin.gov/duke/**PEER REVIEWED**

Milk Concentrations:

Ethyl n-butyrate was identified as one of the volatile compounds from milk(1). [(1) Urbach G; J Chromatogr 404: 163-74 (1987)] **PEER REVIEWED**

As taken from HSDB, 2015.

“Important flavor contributors reported in sweet orange essence include ….. ethyl butyrate….“

As taken from Khan and Abourashed, 2010.

“[Ethyl butyrate] has a role as a plant metabolite”

As taken from PubChem.

3. Status in legislation and other official guidance

Summary of Evaluations Performed by the Joint FAO/WHO Expert Committee on Food Additives ETHYL BUTYRATE	
COE No.:	264
FEMA No.:	2427
JECFA No.:	29
Chemical names:	ETHYL BUTANOATE
Functional class:	FLAVOURING AGENT
Latest evaluation:	1996
ADI:	0-15 mg/kg bw (1967)
Comments:	No safety concern at current levels of intake when used as a flavouring agent. The 1967 ADI of 0-15 mg/kg bw was maintained at the forty-sixth meeting (1996).
Report:	TRS 868-JECFA 46/21

Specifications:	COMPENDIUM ADDENDUM 4/FNP 52 Add.4/177
Tox monograph:	See TRS 868-JECFA 46/64
Previous status:	1967, NMRS 44/TRS 383-JECFA 11/12, FAS 69.31/NMRS 44B-JECFA 11/17 (COMPENDIUM/587), FAS 68.33/NMRS 44A-JECFA 11/25. 0-15. FU. N

As taken from JECFA, 2002 evaluation of Ethyl Butyrate.

Acceptable Daily Intakes:

0-15 MG/KG. [Opdyke, D.L.J. (ed.). Monographs on Fragrance Raw Materials. New York: Pergamon Press, 1979., p. 358] **PEER REVIEWED**

As taken from HSDB, 2015.

Ethyl butyrate is included on the FDA's inventory of "Substances Added to Food (formerly EAFUS)" as a flavoring agent or adjuvant and is generally recognized as safe (GRAS) at defined use levels as a general purpose food additive under 21 CFR Section 182.60 (Synthetic flavoring substances and adjuvants) (FDA, 2022a,b).

There is a REACH dossier on ethyl butyrate (ECHA, undated).

Ethyl butyrate (CAS RN 105-54-4) is listed in the US EPA Toxic Substances Control Act (TSCA) inventory and also in the US EPA 2020 CDR list (Chemical Data Reporting Rule). US EPA 2020 CDR List. US EPA TSCA inventory.

Ethyl butyrate (CAS RN 105-54-4) is listed in the US EPA InertFinder Database (2022) as approved for non-food and fragrance use pesticide products.

Ethyl butyrate is included on the US EPA's list of Safer Chemical Ingredients with functional use: fragrances (US EPA, 2022).

Butanoic acid, ethyl ester (CAS RN 105-54-4) is included on the New Zealand Inventory of Chemicals with HSNO Approval Code HSR001146 (NZ EPA, 2006) and is classified according to the Environmental Protection Authority of New Zealand (NZ EPA CCID).

Classified by the Japanese Ministry of Health, Labour and Welfare (MHLW), Ministry of the Environment MOE) (NITE, 2014).

Ethyl butyrate (CAS RN 105-54-4) is not classified for packaging and labelling under Regulation (EC) No. 1272/2008 (ECHA, 2022).

Ethyl butyrate (CAS RN 105-54-4) is listed as authorised for use as a flavouring substance in all categories of flavoured food in the EU under Regulation (EU) No. 872/2012 (European Commission, 2012).

Ethyl butyrate has been given GRAS (generally recognised as safe) status by FEMA (FEMA no. 2427) (Hall and Oser, 1965).

Ethyl butyrate is included on the US FDA's list of inactive ingredients for approved drug products. It is permitted for use as an ingredient in various products, at the following maximum potency per unit doses and maximum daily exposures:

Inactive ingredient	**Route**	**Dosage form**	**CAS RN**	**UNII**	**Maximum potency per unit dose**	**Maximum Daily Exposure (MDE)**

ETHYL BUTYRATE	ORAL	SOLUTION	105544	UFD2LZ005D		4mg
ETHYL BUTYRATE	ORAL	SUSPENSION	105544	UFD2LZ005D		1mg

As taken from FDA, 2022c.

Butanoic acid, ethyl ester (CAS RN 105-54-4) “poses no unreasonable risk to human health based on Tier I assessment under the NICNAS IMAP assessment framework” and “data available on the function of the chemical indicate that it may be used in cosmetics, but only at low concentrations” (AICIS, 2019).

Ethyl butyrate (CAS RN 105-54-4) included on Health Canada’s Natural Health Products Ingredients Database and can be used as a flavour enhancer in non-medicinal and general oral natural health products. Taking into account the JECFA ADI, there is a toxicity restriction for its use as a flavouring agent of “up to 15mg/kg body weight daily” (Health Canada, 2021).

4. Metabolism/Pharmacokinetics

4.1. Metabolism/metabolites

“This ester is hydrolysed by pancreatic lipase to ethyl alcohol and butyric acid”

As taken from JECFA, 1967 available at http://www.inchem.org/documents/jecfa/jecmono/v44aje10.htm

4.2. Absorption, distribution and excretion

“This compound hydrolyzes into normal constituents of food.”

As taken from JECFA, 1967 available at http://www.inchem.org/documents/jecfa/jecmono/v44aje10.htm

4.3. Interactions

“Although it is well known that food intake is affected by the palatability of food, the actual effect of flavoring on regulation of energy-homeostasis and reward perception by the brain, remains unclear. We investigated the effect of ethyl-butyrate (EB), a common non-caloric food flavoring, on the blood oxygen level dependent (BOLD) response in the hypothalamus (important in regulating energy homeostasis) and ventral tegmental area (VTA; important in reward processes). The 16 study participants (18-25 years, BMI 20-23 kg/m2) drank four study stimuli on separate visits using a crossover design during an fMRI setup in a randomized order. The stimuli were; plain water, water with EB, glucose solution (50gram/300 ml) and glucose solution with EB. BOLD responses to ingestion of the stimuli were determined in the hypothalamus and VTA as a measure of changes in neuronal activity after ingestion. In the hypothalamus and VTA, glucose had a significant effect on the BOLD response but EB flavoring did not. Glucose with and without EB led to similar decrease in hypothalamic BOLD response and glucose with EB resulted in a decrease in VTA BOLD response. Our results suggest that the changes in neuronal activity in the hypothalamus are mainly driven by energy ingestion and EB does not influence the hypothalamic response. Significant changes in VTA neuronal activity are elicited by energy combined with flavor.” As taken from van Opstal AM et al. 2019. Sci. Rep. 9(1), 11250. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/31375749/

5. Toxicity

5.1. Single dose toxicity

Organism	Test Type	Route	Reported Dose (Normalized Dose)	Effect	Source
rabbit	LD50	oral	5228mg/kg (5228mg/kg)		Industrial Medicine and Surgery. Vol. 41, Pg. 31, 1972.
rabbit	LD50	skin	>2gm/kg (2000mg/kg)		Food and Cosmetics Toxicology. Vol. 12, Pg. 719, 1974.
rat	LD50	oral	13gm/kg (13000mg/kg)	BEHAVIORAL: SOMNOLENCE (GENERAL DEPRESSED ACTIVITY) BEHAVIORAL: COMA	Food and Cosmetics Toxicology. Vol. 2, Pg. 327, 1964.

As taken from ChemIDplus, available at https://chem.nlm.nih.gov/chemidplus/

/LABORATORY ANIMALS: Acute Exposure/ Oral admin to dogs of 3 g in 60 mL of water caused no toxic effects ... .[Browning, E. Toxicity and Metabolism of Industrial Solvents. New York: American Elsevier, 1965., p. 580] **PEER REVIEWED**

/LABORATORY ANIMALS: Acute Exposure/ ... In rabbits admin of 2.14 mL/kg caused ... increase in respiratory vol ... intravenous injection, for dogs, 177-222 mg/kg ... had no ... effect ... .[Browning, E. Toxicity and Metabolism of Industrial Solvents. New York: American Elsevier, 1965., p. 580] **PEER REVIEWED**

As taken from HSDB, 2015

Animal	Route	LD50 (mg/kg bw)	References
Rat	oral	13050	Jenner et al., 1964

JECFA (1967)

5.2. Repeated dose toxicity

Rat. In a feeding experiment on 15 males and 15 females for 12 weeks no adverse effect was noted at 14.4 mg/kg body-weight/day (Oser, 1967).

As taken from JECFA, 1967 available at http://www.inchem.org/documents/jecfa/jecmono/v44aje10.htm

5.3. Reproduction toxicity

The potential of ethyl n-butyrate to cause infertility, abortions, or birth defects has not been examined in laboratory animals.

As taken from HSDB, 2015.

Quantitative Risk Type; Not calculated

Quantitative Risk Value; Not calculated

Product Use; Not specified

Safety Evaluation Owner ; COSMOS TTC (NON-CANCER)

POD Method; NOEL

POD Value; 4.8

POD Owner ; COSMOS TTC (NON-CANCER)

Adjustment factors used in calculations: Adjustment factor: Study: Dose Duration: 3.0 (3)

Critical study: RAT (Reproductive/Developmental Toxicity) Oral - dietary exposure for 1 GEN

NOEL/LEL Owner; MUNRO

Original NOEL ; 14.4 mg/kg bw/day

Original LEL; Not established

Critical Sites; [not given]

Critical Effects; • NO EFFECTS

Safety Evaluation Comments: no comments available.

Source Document: no source document available

As taken from the COSMOS database available at https://ng.cosmosdb.eu

5.4. Mutagenicity

Test System:	AMES SALMONELLA TYPHIMURIUM
Strain Indicator:	TA97
Metabolic Activation:	NONE
Method:	PREINCUBATION

Dose:	0.01-1 MG/PLATE (TEST MATERIAL SOLVENT: DMSO)
Results:	NEGATIVE
Reference:	[FUJITA,H, SUMI,C AND SASAKI,M; MUTAGENICITY TEST OF FOOD ADDITIVES WITH SALMONELLA TYPHIMURIUM TA97 AND TA102. VII;KENKYU NENPO - TOKYO-TORITSU EISEI KENKYUSHO 43:219-227, 1992]
Test System:	AMES SALMONELLA TYPHIMURIUM
Strain Indicator:	TA97
Metabolic Activation:	RAT, LIVER, S-9, AROCLOR 1254
Method:	PREINCUBATION
Dose:	0.01-1 MG/PLATE (TEST MATERIAL SOLVENT: DMSO)
Results:	NEGATIVE
Reference:	[FUJITA,H, SUMI,C AND SASAKI,M; MUTAGENICITY TEST OF FOOD ADDITIVES WITH SALMONELLA TYPHIMURIUM TA97 AND TA102. VII;KENKYU NENPO - TOKYO-TORITSU EISEI KENKYUSHO 43:219-227, 1992]
Test System:	AMES SALMONELLA TYPHIMURIUM
Strain Indicator:	TA102
Metabolic Activation:	NONE
Method:	PREINCUBATION
Dose:	0.01-1 MG/PLATE (TEST MATERIAL SOLVENT: DMSO)
Results:	NEGATIVE
Reference:	[FUJITA,H, SUMI,C AND SASAKI,M; MUTAGENICITY TEST OF FOOD ADDITIVES WITH SALMONELLA TYPHIMURIUM TA97 AND TA102. VII;KENKYU NENPO - TOKYO-TORITSU EISEI KENKYUSHO 43:219-227, 1992]
Test System:	AMES SALMONELLA TYPHIMURIUM
Strain Indicator:	TA102
Metabolic Activation:	RAT, LIVER, S-9, AROCLOR 1254
Method:	PREINCUBATION
Dose:	0.01-1 MG/PLATE (TEST MATERIAL SOLVENT: DMSO)

Results:	NEGATIVE

As taken from CCRIS, 1995.

Test system	Test conditions	Endpoint	Activation status	Results	Reference
Salmonella typhimurium strains TA98 and TA100	Ames test. Tested at concentrations of up to 10 mg/plate.	Mutation	With and without	-ve (Limited study, only two strains tested)	NTP, 2007
Escherichia coli strain pKM101	Tested at concentrations of up to 10 mg/plate.	Mutation	With and without	-ve	NTP, 2007

The Ames test was used to evaluate the mutagenicity of a number of neat complex flavor mixtures. Studies in which ethyl butyrate was part of the test mixture include EMT000313 (CD-ROM 1, JTI Submission, 2002). The results show that these mixtures were not mutagenic.

Test system	Test conditions	Endpoint	Activation	Result	Reference
Salmonella typhimurium TA92, TA94, TA98, TA100, TA1535, TA1537 and possibly TA2637	Ames test at up to a maximum dose of 10 mg/plate	Mutation	With and without S9	-ve Good quality study	Ishidate et al. 1984
Salmonella typhimurium strains TA97, TA102	Up to 1 mg/plate [Limited study; only two strains tested – but extends the range of strains tested – see Ishidate et al. 1984 above].	Mutation	With and without S9	-ve	Fujita et al. 1992

Other in vitro Test:

Test system	Test conditions	Endpoint	Activation	Result	Reference
Chinese hamster ovary cells	Incubated for 48 hr at up to 2 mg/ml (dose previously found to inhibit cell growth by 50%). Assessed chromosome aberrations	Chromosome damage	Without	-ve (limited study, not tested with S9)	Ishidate et al. 1984

To screen for inositol-depleting valproate-like compounds as potential mood stabilizing drugs. MAIN METHODS: We exploited the yeast Saccharomyces cerevisiae, as a model in which inositol de novo synthesis has been extensively characterized, to test the effects of ethyl butyrate (EB), 2-ethyl-butyric acid, sodium butyrate, and n-propyl hexanoate on inositol biosynthesis. Cell growth was followed by measuring the optical density of the cultures (spectrophotometrically), RNA abundance was determined by Northern blot analysis, intracellular inositol was measured by a fluorometric assay, and 1-d-myo-inositol-3-phosphate synthase activity was examined using a

chromatographic method. KEY FINDINGS: Of the tested compounds, only EB exhibited an inositol-depleting effect. The inositol-depleting effect of EB was achieved without significant adverse effect on cell growth, pointing to lesser toxicity compared to valproate. SIGNIFICANCE: These results indicate that EB is a potential candidate for mood-stabilizing therapy. Azab AN; Mehta DV; Chesebro JE; Greenberg ML. Life Sci. 2009, Jan 2; 84(1-2):38-44. [Life sciences] [PubMed] available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=retrieve&db=pubmed&list_uids=19028504&dopt=AbstractPlus

5.5. Cytotoxicity

Toxicity threshold (cell multiplication inhibition test): bacteria (Pseudomonas putida): 140 mg/l (Bringmann & Kühn 1980a)

As taken from Finnish Environment Institute

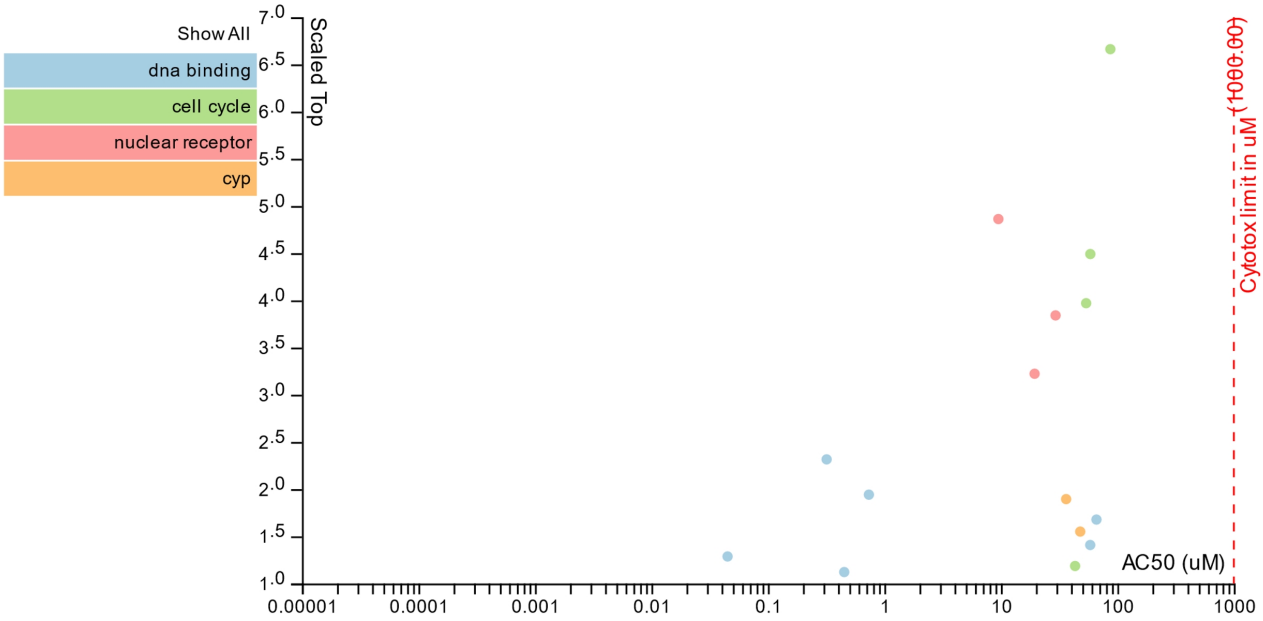
High-throughput Assay Data

The US Environmental Protection Agency (EPA) evaluated ethyl butyrate in a series of high-throughput assays, which are publicly available on the US EPA's CompTox Dashboard (section BIOACTIVITY / sub-section TOXCAST:SUMMARY), available at the following URL: https://comptox.epa.gov/dashboard

US EPA provides the following data use considerations for ToxCast data: "The activity of a chemical in a specific assay does not necessarily mean that it will cause toxicity or an adverse health outcome. There are many factors that determine whether a chemical will cause a specific adverse health outcome. Careful review is required to determine the use of the data in a particular decision contexts. Interpretation of ToxCast data is expected to change over time as both the science and analytical methods improve."

A summary of the ToxCast assay data on ethyl butyrate is provided below in Figure 1. Figure 1 proves an overview of the types of assays where activity was noted with this substance. The complete study details are available on US EPA's CompTox Dashboard.

Figure 1



“E-cigarettes utilize a wide range of flavoring chemicals with respiratory health effects that are not well understood. In this study, we used pulmonary-associated cell lines to assess the in vitro cytotoxic effects of 30 flavoring chemicals. Human bronchial epithelial cells (BEAS-2B) and both naïve and activated macrophages (THP-1) were treated with 10, 100, and 1000 μM of flavoring chemicals and analyzed for changes in viability, cell membrane damage, reactive oxygen species (ROS) production, and inflammatory cytokine release. Viability was unaffected for all chemicals at the 10 and 100 μM concentrations. At 1000 μM, the greatest reductions in viability were seen with decanal, hexanal, nonanal, cinnamaldehyde, eugenol, vanillin, alpha-pinene, and limonene. High amounts of ROS were elicited by vanillin, ethyl maltol, and the diketones (2,3-pentanedione, 2,3-heptanedione, and 2,3-hexanedione) from both cell lines. Naïve THP-1 cells produced significantly elevated levels of IL-1β, IL-8, and TNF-α when exposed to ethyl maltol and hexanal. Activated THP-1 cells released increased IL-1β and TNF-α when exposed to ethyl maltol, but many flavoring chemicals had an apparent suppressive effect on inflammatory cytokines released by activated macrophages, some with varying degrees of accompanying cytotoxicity. The diketones, L-carvone, and linalool suppressed cytokine release in the absence of cytotoxicity. These findings provide insight into lung cell cytotoxicity and inflammatory cytokine release in response to flavorings commonly used in e-cigarettes.”

Morris AM et al. (2021) Effects of E-Cigarette Flavoring Chemicals on Human Macrophages and Bronchial Epithelial Cells.

5.6. Carcinogenicity

The potential of on ethyl n-butyrate to cause cancer in laboratory animals has not been examined. The potential for ethyl n-butyrate to cause cancer in humans has not been assessed by the U.S. EPA IRIS program, the International Agency for Research on Cancer, or the U.S. National Toxicology Program 13th Report on Carcinogens.

As taken from HSDB, 2015

5.7. Irritation/immunotoxicity

Skin, Eye and Respiratory Irritations:

Vapor: Irritating to eyes, nose and throat. Liquid: Irritating to skin and eyes. [U.S. Coast Guard, Department of Transportation. CHRIS - Hazardous Chemical Data. Volume II. Washington, D.C.: U.S. Government Printing Office, 1984-5.] **PEER REVIEWED**

As taken from HSDB, 2015.

Ethyl butyrate applied full strength to intact or abraded rabbit skin for 24 hours under occlusion was moderately irritating (Moreno 1972).

Tested at 5% in petrolatum, it produced no irritation after a 48-hr closed-patch test in human subjects (Kligman 1972).

Type of test	Route of exposure	Species observed	Dose data	Reaction severity	Reference
Standard Draize test	Administration onto the skin	Rodent – rabbit	500 mg/24H	Moderate	FCTXAV Food and Cosmetics Toxicology. (London, UK) V.1-19, 1963-81. For publisher information, see FCTOD7. Volume(issue)/page/year: 12,719,1974

As taken from RTECS, 1997.

Sensitization:

No sensitization reactions were induced in 25 volunteers following a maximization test with a concentration of 5% in petrolatum (Kligman 1972). [This procedure usually involves five 48-hr covered patch tests (often separated by 24 hr) followed 10-14 days later by a 48-hr covered challenge patch test using the same concentration].

Ethyl butyrate is a suspected skin sensitiser. The CAESAR skin sensitization model in the VEGA (Q)SAR platform predicts that the chemical is a sensitizer (good reliability).

As taken from ECHA, 2016.

The reliability and applicability of this QSAR prediction as standalone source of toxicological information is limited and inappropriate for some complex endpoints like reprotoxicity or carcinogenicity. Nevertheless, for the toxicological assessment of this ingredient, this result was still taken into consideration and used within the WoE approach as a supportive tool, in combination with other sources of information when available, like experimental data or appropriate read-across.

5.8. All other relevant types of toxicity

Total particulate matter (TPM) from heated (tobacco or nicotine) product(s) containing Ethyl Butyrate was tested in a battery of in vitro and/or in vivo test(s). Within the sensitivity and specificity of the bioassay(s) the activity of the TPM was not increased by the addition of Ethyl Butyrate when compared to TPM from 3R4F cigarettes. The table below provides tested level(s) and specific endpoint(s).

Endpoint	**Tested level (ppm)**	**Reference**
In vitro genotoxicity	286	JTI KB Study Report(s)
In vitro cytotoxicity	286	JTI KB Study Report(s)

EXPL THER /The purpose of this study was/ to screen for inositol-depleting valproate-like compounds as potential mood stabilizing drugs. We exploited the yeast Saccharomyces cerevisiae, as a model in which inositol de novo synthesis has been extensively characterized, to test the effects of ethyl butyrate (EB), 2-ethyl-butyric acid, sodium butyrate, and n-propyl hexanoate on inositol biosynthesis. Cell growth was followed by measuring the optical density of the cultures (spectrophotometrically), RNA abundance was determined by Northern blot analysis, intracellular inositol was measured by a fluorometric assay, and 1-d-myo-inositol-3-phosphate synthase activity was examined using a chromatographic method. Of the tested compounds, only EB exhibited an inositol-depleting effect. The inositol-depleting effect of EB was achieved without significant adverse effect on cell growth, pointing to lesser toxicity compared to valproate. These results indicate that EB is a potential candidate for mood-stabilizing therapy. [Azab AN et al; Life Sci 84 (1-2): 38-44 (2009)] **PEER REVIEWED** PubMed Abstract

As taken from HSDB, 2015

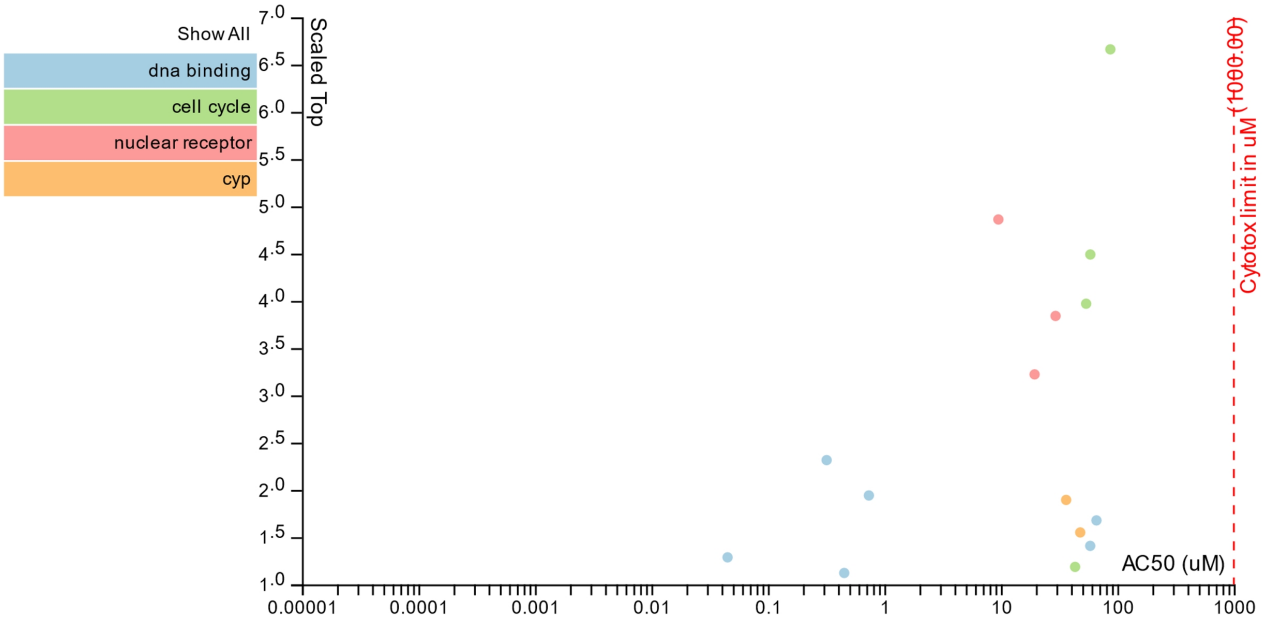
High-throughput Assay Data

The US Environmental Protection Agency (EPA) evaluated ethyl butyrate in a series of high-throughput assays, which are publicly available on the US EPA's CompTox Dashboard (section BIOACTIVITY / sub-section TOXCAST:SUMMARY), available at the following URL: https://comptox.epa.gov/dashboard

US EPA provides the following data use considerations for ToxCast data: "The activity of a chemical in a specific assay does not necessarily mean that it will cause toxicity or an adverse health outcome. There are many factors that determine whether a chemical will cause a specific adverse health outcome. Careful review is required to determine the use of the data in a particular decision contexts. Interpretation of ToxCast data is expected to change over time as both the science and analytical methods improve."

A summary of the ToxCast assay data on ethyl butyrate is provided below in Figure 1. Figure 1 proves an overview of the types of assays where activity was noted with this substance. The complete study details are available on US EPA's CompTox Dashboard.

Figure 1



6. Functional effects on

6.1. Broncho/pulmonary system

Skin, Eye and Respiratory Irritations:

Vapor: Irritating to eyes, nose and throat. Liquid: Irritating to skin and eyes. [U.S. Coast Guard, Department of Transportation. CHRIS - Hazardous Chemical Data. Volume II. Washington, D.C.: U.S. Government Printing Office, 1984-5.] **PEER REVIEWED**

Tested at 5% in petrolatum, ethyl butyrate produced no irritation after a 48 hr closed-patch test in 25 human subjects. A maximization test ... on 25 volunteers at a concentration of 5% in petrolatum ... produced no sensitization reactions. /Butyrate/ [Opdyke, D.L.J. (ed.). Monographs on Fragrance Raw Materials. New York: Pergamon Press, 1979., p. 353] **PEER REVIEWED**

In rabbits admin of 2.14 mL/kg caused an increase in respiratory volume.

As taken from HSDB, 2015

6.2. Cardiovascular system

“IN VITRO IT HAS BEEN SHOWN TO HAVE A HEMOLYTIC EFFECT SLIGHTLY GREATER THAN THAT OF METHYL BUTYRATE.”

As taken from HSDB, 2015.

6.3. Nervous system

“Inhalation of high concentrations causes narcotic effects.”

“Inhalation of high concentrations can cause CNS depression”

“Neurotoxin: Acute solvent syndrome”

“Occupational diseases associated with exposure to this agent: Encephalopathy, chronic solvent”

As taken from Haz-Map, 2020.

6.4. Other organ systems, dependent on the properties of the substance

“Headache , nausea, vomiting and lightheadedness can occur from exposure to ethyl n-butyrate over a long time.”

Loss of consciousness can occur at high dose.

As taken from HSDB, 2015

7. Addiction

JTI is not aware of any information that demonstrates that this ingredient has any addictive effect.

8. Burnt ingredient toxicity

This ingredient was considered as part of an overall safety assessment of ingredients added to tobacco in the manufacture of cigarettes. An expert panel of toxicologists reviewed the open literature and internal toxicology data of 5 tobacco companies to evaluate a composite list of ingredients used in the manufacture of cigarettes. The conclusion of this report was that these

ingredients did not increase the inherent biological activity of tobacco cigarettes, and are considered to be acceptable under conditions of intended use (Doull et al., 1994 & 1998).

Tobacco smoke condensates from cigarettes containing ethyl butyrate and an additive free, reference cigarettes were tested in a battery of in vitro and/or in vivo test(s). Within the sensitivity and specificity of the bioassay(s) the activity of the condensate was not changed by the addition of ethyl butyrate. Table below provides tested level(s) and specific endpoint(s).

Endpoint	Tested level (ppm)	Reference
Smoke chemistry	28	Carmines, 2002 & Rustemeier et al. 2002
	292	Baker et al. 2004a
	13 390 1100 (Cigar)	JTI KB Study Report(s)
	8190	Roemer et al., 2014
In vitro genotoxicity	28	Carmines, 2002 & Röemer et al. 2002
	292	Baker et al. 2004c
	13	Renne et al. 2006
	13 390 1100 (Cigar)	JTI KB Study Report(s)
	493	fGLH Study Report (2010)
	8190	Roemer et al., 2014
In vitro cytotoxicity	28	Carmines, 2002 & Röemer et al. 2002
	292	Baker et al. 2004c
	390 1100 (Cigar)	JTI KB Study Report(s)
	493	fGLH Study Report (2010)
	8190	Roemer et al., 2014
Inhalation study	<0.1	Gaworski et al. 1998
	28	Carmines, 2002 & Vanscheeuwijck et al. 2002
	292	Baker et al. 2004c
	13	Renne et al. 2006
	13 390	JTI KB Study Report(s)
	8190	Schramke et al., 2014
Skin painting	13 390	JTI KB Study Report(s)
In vivo genotoxicity	8190 1100 (cigar)	Schramke et al., 2014 JTI KB Study Report(s)

Transfer studies:

In a pyrolysis study, 98% of ethyl butyrate added to cigarettes was transferred intact to the smoke (Purkis et al. 2011).

9. Heated/vapor emissions toxicity

Total particulate matter (TPM) from heated (tobacco or nicotine) product(s) containing Ethyl Butyrate was tested in a battery of in vitro and/or in vivo test(s). Within the sensitivity and specificity of the bioassay(s) the activity of the TPM was not increased by the addition of Ethyl Butyrate when

compared to TPM from 3R4F cigarettes. The table below provides tested level(s) and specific endpoint(s).

Endpoint	Tested level (ppm)	Reference
In vitro genotoxicity	286	JTI KB Study Report(s)
In vitro cytotoxicity	286	JTI KB Study Report(s)

Aerosol from an electronic nicotine delivery system (ENDS) that creates a vapor by heating an e-liquid containing Ethyl butyrate was tested in a battery of in vitro and/or in vivo test(s). Under the test conditions and within the sensitivity and specificity of the bioassay(s), no mutagenic, genotoxic or cytotoxic responses were observed when exposed to Aerosol Collected Matter (ACM) and/or aerosol Gas Vapor Phase (GVP) and no adverse findings from a 90-day in vivo repeat-dose inhalation toxicity study were observed after exposure to the aerosol even when exposure concentrations were the maximal amount that could be achieved with the specific product(s). These results are in contrast to those observed with combustible cigarette which showed mutagenic, genotoxic, cytotoxic and adverse effects upon exposure. The table below provides the highest tested level(s) and specific endpoint(s):

Endpoint	Tested level (ppm)	Reference
Aerosol chemistry	2,500	Logic (2019a) Labstat International Inc. (2021)
In vitro genotoxicity	2,500	Logic (2019a) Labstat International Inc. (2022)
In vitro cytotoxicity	2,500	Logic (2019a) Labstat International Inc. (2022)
In vivo genotoxicity	2,500	Logic (2019a)
Inhalation study	2,500	Logic (2019a)

Aerosol from an electronic nicotine delivery system (ENDS) product that creates a vapor by heating an e-liquid; the vapor then passes through a capsule containing tobacco granules, containing Ethyl butyrate was tested in a battery of in vitro and/or in vivo test(s). Under the test conditions and within the sensitivity and specificity of the bioassay(s), no mutagenic, genotoxic or cytotoxic responses were observed when exposed to Aerosol Collected Matter (ACM) and/or aerosol Gas Vapor Phase (GVP) and no adverse findings from a 90-day in vivo repeat-dose inhalation toxicity study were observed after exposure to the aerosol even when exposure concentrations were the maximal amount that could be achieved with the specific product(s). These results are in contrast to those observed with combustible cigarette which showed mutagenic, genotoxic, cytotoxic and adverse effects upon exposure. The table below provides tested level(s) and specific endpoint(s):

Endpoint	Tested level	Reference
Aerosol chemistry	0.297 mg/(tobacco portion; 310 mg)	Logic (2019b)
In vitro genotoxicity	0.297 mg/(tobacco portion; 310 mg)	Logic (2019b)
In vitro cytotoxicity	0.297 mg/(tobacco portion; 310 mg)	Logic (2019b)
In vivo genotoxicity	0.297 mg/(tobacco portion; 310 mg)	Logic (2019b)

Inhalation study	0.297 mg/(tobacco portion; 310 mg)	Logic (2019b)

Aerosol from heated tobacco stick(s) containing Ethyl butyrate was tested in aerosol chemistry and a battery of in vitro test(s). Under the test conditions and within the sensitivity and specificity of the bioassay(s), the activity of the total particulate matter (TPM) and/or gas vapor phase (GVP) were not increased by the addition of this ingredient when compared to TPM and/or GVP from reference combustible cigarettes. The table below provides the highest tested level(s) and specific endpoint(s):

Endpoint	Tested level (mg/stick)	Reference
Aerosol chemistry	3.55	Labstat International Inc. (2020a) Labstat International Inc. (2021a)
In vitro genotoxicity	3.55	Labstat International Inc. (2020b) Labstat International Inc. (2021b)
In vitro cytotoxicity	3.55	Labstat International Inc. (2020b) Labstat International Inc. (2021b)

“The widespread use of electronic cigarettes (e-cig) is a serious public health concern; however, mechanisms by which e-cig impair the function of airway epithelial cells-the direct target of e-cig smoke-are not fully understood. Here we report transcriptomic changes, including decreased expression of many ribosomal genes, in airway epithelial cells in response to e-cig exposure. Using RNA-seq we identify over 200 differentially expressed genes in air-liquid interface cultured primary normal human bronchial epithelial (NHBE) exposed to e-cig smoke solution from commercial e-cig cartridges. In particular, exposure to e-cig smoke solution inhibits biological pathways involving ribosomes and protein biogenesis in NHBE cells. Consistent with this effect, expression of corresponding ribosomal proteins and subsequent protein biogenesis are reduced in the cells exposed to e-cig. Gas chromatography/mass spectrometry (GC/MS) analysis identified the presence of five flavoring chemicals designated as 'high priority' in regard to respiratory health, and methylglyoxal in e-cig smoke solution. Together, our findings reveal the potential detrimental effect of e-cig smoke on ribosomes and the associated protein biogenesis in airway epithelium. Our study calls for further investigation into how these changes in the airway epithelium contribute to the current epidemic of lung injuries in e-cig users.”

Hae-Ryung P et al. (2021) Electronic cigarette smoke reduces ribosomal protein gene expression to impair protein synthesis in primary human airway epithelial cells.

10. Ecotoxicity

10.1. Environmental fate

If ethyl n-butyrate is released to air, it will be broken down by reaction with other chemicals. It is not broken down by light. If released to water or soil, is not expected to bind to soil particles or suspended particles. Ethyl n-butyrate is expected to move through soil. Ethyl n-butyrate is expected to move into air from wet soils or water surfaces. Ethyl n-butyrate may be broken down by microorganisms and may build up in tissues of aquatic organisms.

Environmental Fate/Exposure Summary:

Ethyl n-butyrate's production and use in manufacturing artificial rum, in perfumery, as a flavoring ingredient and as a solvent may result in its release to the environment through various waste streams. Ethyl n-butyrate occurs naturally in some plants and is produced from garden and household waste. If released to air, a vapor pressure of 14.0 mm Hg at 25 deg C indicates ethyl n-

butyrate will exist solely as a vapor in the atmosphere. Vapor-phase ethyl n-butyrate will be degraded in the atmosphere by reaction with photochemically-produced hydroxyl radicals; the half-life for this reaction in air is estimated to be 3 days. Ethyl n-butyrate does not contain chromophores that absorb at wavelengths >290 nm and, therefore, is not expected to be susceptible to direct photolysis by sunlight. If released to soil, ethyl n-butyrate is expected to have very high mobility based upon an estimated Koc of 20. Volatilization from moist soil surfaces is expected to be an important fate process based upon an estimated Henry's Law constant of 4.4X10-4 atm-cu m/mole. Ethyl n-butyrate has been classified as readily biodegradable as estimated by results from analogous compounds in the Japanese MITI test, suggesting that biodegradation may be an important environmental fate process in soil and water. If released into water, ethyl n-butyrate is not expected to adsorb to suspended solids and sediment based upon the estimated Koc. Volatilization from water surfaces is not expected to be an important fate process based upon this compound's estimated Henry's Law constant. Estimated volatilization half-lives for a model river and model lake are 5 hrs and 5 days, respectively. An estimated BCF of 8 suggests the potential for bioconcentration in aquatic organisms is low. Hydrolysis is not expected to be an important environmental fate process with base-catalyzed second-order hydrolysis half-lives of 3.5 years and 130 days at pH values of 7 and 8, respectively. Occupational exposure to ethyl n-butyrate may occur through inhalation and dermal contact with this compound at workplaces where ethyl n-butyrate is produced or used. Monitoring data indicate that the general population may be exposed to n-ethyl butyrate via ingestion of food and dermal contact with consumer products containing ethyl n-butyrate.(SRC)

Artificial Pollution Sources:

Ethyl n-butyrate's production and use in manufacturing artificial rum, in perfumery(1), as a flavoring ingredient and as a solvent(2) may result in its release to the environment through various waste streams(SRC). [(1) O'Neil MJ, ed; The Merck Index. 15th ed., Cambridge, UK: Royal Society of Chemistry, p. 699 (2013) (2) Lewis RJ Sr; Hawley's Condensed Chemical Dictionary. 15th ed., New York, NY: John Wiley & Sons, Inc., p. 521 (2007)] **PEER REVIEWED**

Environmental Fate:

TERRESTRIAL FATE: Based on a classification scheme(1), an estimated Koc value of 20(SRC), determined from a structure estimation method(2), indicates that ethyl nbutyrate is expected to have very high mobility in soil(SRC). Volatilization of ethyl n-butyrate from moist soil surfaces is expected to be an important fate process(SRC) given an estimated Henry's Law constant of 4.4X10-4 atm-cu m/mole(SRC), based upon its vapor pressure, 14.0 mm Hg(3), and water solubility, 4,900 mg/L(4). Ethyl n-butyrate is expected to volatilize from dry soil surfaces(SRC) based upon its vapor pressure(3). Ethyl n-butyrate has been classified as readily biodegradable as estimated by results from analogous compounds in the Japanese MITI test(5), suggesting that biodegradation may be an important environmental fate process in soil(SRC). [(1) Swann RL et al; Res Rev 85: 17-28 (1983) (2) US EPA; Estimation Program Interface (EPI) Suite. Ver. 4.1. Nov, 2012. Available from, as of Jun 4, 2015: http://www.epa.gov/oppt/exposure/pubs/episuitedl.htm (3) Daubert TE, Danner RP; Physical and Thermodynamic Properties of Pure Compounds Am Inst Chem Eng (1995) (4) Riddick JA et al; Organic Solvents New York, NY: John Wiley & Sons Inc. (1984) (5) NITE; Chemical Risk Information Platform (CHRIP). Biodegradation and Bioconcentration. Tokyo, Japan: Natl Inst Tech Eval. Available from, as of Jun 5, 2015: http://www.safe.nite.go.jp/english/db.html **PEER REVIEWED**

AQUATIC FATE: Based on a classification scheme(1), an estimated Koc value of 20(SRC), determined from a structure estimation method(2), indicates that ethyl n-butyrate is not expected to adsorb to suspended solids and sediment(SRC). Volatilization from water surfaces is expected(3) based upon an estimated Henry's Law constant of 4.4X10-4 atm-cu m/mole(SRC), derived from its vapor pressure, 14.0 mm Hg(4), and water solubility, 4,900 mg/L(5). Using this Henry's Law constant and an estimation method(3), volatilization half-lives for a model river and model lake are 5 hrs and 5 days, respectively(SRC). According to a classification scheme(6), an estimated BCF of

8(SRC), from an estimated log Kow of 1.85(2) and a regression-derived equation(2), suggests the potential for bioconcentration in aquatic organisms is low(SRC). Ethyl n-butyrate has been classified as readily biodegradable as estimated by results from analogous compounds in the Japanese MITI test(7), suggesting that biodegradation may be an important environmental fate process in water(SRC). [(1) Swann RL et al; Res Rev 85: 17-28 (1983) (2) US EPA; Estimation Program Interface (EPI) Suite. Ver. 4.1. Nov, 2012. Available from, as of Jun 5, 2015: http://www.epa.gov/oppt/exposure/pubs/episuitedl.htm (3) Lyman WJ et al; Handbook of Chemical Property Estimation Methods. Washington, DC: Amer Chem Soc pp. 15-1 to 15-29 (1990) (4) Daubert TE, Danner RP; Physical and Thermodynamic Properties of Pure Compounds Am Inst Chem Eng (1995) (5) Riddick JA et al; Organic Solvents New York, NY: John Wiley & Sons Inc. (1984) (6) Franke C et al; Chemosphere 29: 1501-14 (1994) (7) NITE; Chemical Risk Information Platform (CHRIP). Biodegradation and Bioconcentration. Tokyo, Japan: Natl Inst Tech Eval. Available from, as of Jun 5, 2015: http://www.safe.nite.go.jp/english/db.html **PEER REVIEWED**

ATMOSPHERIC FATE: According to a model of gas/particle partitioning of semivolatile organic compounds in the atmosphere(1), ethyl n-butyrate, which has a vapor pressure of 14 mm Hg at 25 deg C(2), is expected to exist solely as a vapor in the ambient atmosphere. Vapor-phase ethyl n-butyrate is degraded in the atmosphere by reaction with photochemically-produced hydroxyl radicals(SRC); the half-life for this reaction in air is estimated to be 3 days(SRC), calculated from its rate constant of 4.94X10-12 cu cm/molecule-sec at 25 deg C(3). Ethyl n-butyrate does not contain chromophores that absorb at wavelengths >290 nm(4) and, therefore, is not expected to be susceptible to direct photolysis by sunlight(SRC). [(1) Bidleman TF; Environ Sci Technol 22: 361-367 (1988) (2) Daubert TE, Danner RP; Physical and Thermodynamic Properties of Pure Compounds Am Inst Chem Eng (1995) (3) Kwok ESC, Atkinson R; Estimation of hydroxyl radical reaction rate constants for gas-phase organic compounds using a structure-reactivity relationship: an update. Riverside, CA: Univ CA, Statewide Air Pollut Res Ctr., CMA Contract No. AFC-8.0-OR (1994) (4) Lyman WJ et al; Handbook of Chemical Property Estimation Methods. Washington, DC: Amer Chem Soc pp. 8-12 (1990)] **PEER REVIEWED**

Environmental Biodegradation:

AEROBIC: Ethyl n-butyrate has been classified as readily biodegradable as estimated by results from analogous compounds in the Japanese MITI test(1). [(1) NITE; Chemical Risk Information Platform (CHRIP). Biodegradation and Bioconcentration. Tokyo, Japan: Natl Inst Tech Eval. Available from, as of Jun 5, 2015: http://www.safe.nite.go.jp/english/db.html **PEER REVIEWED**

Environmental Abiotic Degradation:

The rate constant for the vapor-phase reaction of ethyl n-butyrate with photochemically-produced hydroxyl radicals is 4.94X10-12 cu cm/molecule-sec at 25 deg C(1). This corresponds to an atmospheric half-life of about 3 days at an atmospheric concentration of 5X10+5 hydroxyl radicals per cu cm(1). A base-catalyzed second-order hydrolysis rate constant of 6.3 L/mole-sec(SRC) was estimated using a structure estimation method(2); this corresponds to half-lives of 3.5 years and 130 days at pH values of 7 and 8, respectively(2). Ethyl n-butyrate does not contain chromophores that absorb at wavelengths >290 nm(3) and, therefore, is not expected to be susceptible to direct photolysis by sunlight(SRC). [(1) Kwok ESC, Atkinson R; Estimation of hydroxyl radical reaction rate constants for gas-phase organic compounds using a structure-reactivity relationship: an update. Riverside, CA: Univ CA, Statewide Air Pollut Res Ctr., CMA Contract No. AFC-8.0-OR (1994) (2) US EPA; Estimation Program Interface (EPI) Suite. Ver. 4.1. Nov, 2012. Available from, as of Jun 5, 2015: http://www.epa.gov/oppt/exposure/pubs/episuitedl.htm (3) Lyman WJ et al; Handbook of Chemical Property Estimation Methods. Washington, DC: Amer Chem Soc pp. 8-12 (1990)] **PEER REVIEWED**

Environmental Bioconcentration:

An estimated BCF of 8 was calculated in fish for ethyl n-butyrate(SRC), using an estimated log Kow of 1.85(1) and a regression-derived equation(1). According to a classification scheme(2), this BCF

suggests the potential for bioconcentration in aquatic organisms is low(SRC). [(1) US EPA; Estimation Program Interface (EPI) Suite. Ver. 4.1. Nov, 2012. Available from, as of May 13, 3015: http://www.epa.gov/oppt/exposure/pubs/episuitedl.htm/ (2) Franke C et al; Chemosphere 29: 1501-14 (1994)] **PEER REVIEWED**

Soil Adsorption/Mobility:

Using a structure estimation method based on molecular connectivity indices(1), the Koc of ethyl n-butyrate can be estimated to be 20(SRC). According to a classification scheme(2), this estimated Koc value suggests that ethyl n-butyrate is expected to have very high mobility in soil. [(1) US EPA; Estimation Program Interface (EPI) Suite. Ver. 4.1. Nov, 2012. Available from, as of May 13, 2015: http://www.epa.gov/oppt/exposure/pubs/episuitedl.htm (2) Swann RL et al; Res Rev 85: 17-28 (1983)] **PEER REVIEWED**

Volatilization from Water/Soil:

The Henry's Law constant for ethyl n-butyrate is estimated as 4.4X10-4 atm-cu m/mole(SRC) derived from its vapor pressure, 14.0 mm Hg(1), and water solubility, 4,900 mg/L(2). This Henry's Law constant indicates that ethyl n-butyrate is expected to volatilize from water surfaces(3). Based on this Henry's Law constant, the volatilization half-life from a model river (1 m deep, flowing 1 m/sec, wind velocity of 3 m/sec)(3) is estimated as 5 hours(SRC). The volatilization half-life from a model lake (1 m deep, flowing 0.05 m/sec, wind velocity of 0.5 m/sec)(3) is estimated as 5 days(SRC). Ethyl n-butyrate's estimated Henry's Law constant indicates that volatilization from moist soil surfaces may occur(SRC). The potential for volatilization of ethyl n-butyrate from dry soil surfaces may exist(SRC) based upon its vapor pressure(1). [(1) Daubert TE, Danner RP; Physical and Thermodynamic Properties of Pure Compounds Am Inst Chem Eng (1995) (2) Riddick JA et al; Organic Solvents New York, NY: John Wiley & Sons Inc. (1984) (3) Lyman WJ et al; Handbook of Chemical Property Estimation Methods. Washington, DC: Amer Chem Soc pp. 15-1 to 15-29 (1990)] **PEER REVIEWED**

Environmental Water Concentrations:

SURFACE WATER: Ethyl n-butyrate was detected, not quantified in one of eight weakly polluted small rivers and brooks in southwest Germany which flow into Lake Constance(1). [(1) Juettner F; Water Sci Technol 25: 155-64 (1992)] **PEER REVIEWED**

Effluent Concentrations:

Ethyl n-butyrate was detected not quantified in 9 year old leachate from a municipal waste disposal site in Ambt-Delden and Wijster, The Netherlands. The Ambt-Delden leachate was in the acidification stage (pH 5.7, BOD 30,000 mg/L O2). It was not detected in 2 year old leachate from municipal waste disposal site (Wijster, The Netherlands) which was in the methane fermentation stage (pH 7.0, BO 50 mg/L O2)(1). The compound was present at 0.08 ug/cu m in the volatile components of emissions of a European waste incineration plant(2). Ethyl n-butyrate was released during composting of food residue, yard trimmings, agricultural and wood waste at a composting operation in Joyceville, Ontario, Canada, monitored between May and July 1996(3). Ethyl n-butyrate was present at <5 ppbv in the volatiles recovered at 30, 50, and 70 cm depth from the Case Passerini landfill, Florence, Italy(4). [(1) Harmsen J; Water Res 17: 699-705 (1983) (2) Jay K, Stieglitz L; Chemosphere 30: 1249-60 (1995) (3) Krzymiem M et al; J Air Waste Manage Assoc 49: 804-13 (1999) (4) Tassi F et al; Sci Total Environ 407(15): 4513-25 (2009)] **PEER REVIEWED**

As taken from HSDB, 2015.

The Ecological Categorization Results from the Canadian Domestic Substances List state that ethyl butyrate is persistent in the environment:

Media of concern leading to Categorization	Air-Water

Experimental Biodegradation half-life (days)	Not Available
Predicted Ultimate degradation half-life (days)	15
MITI probability of biodegradation	0.9501
TOPKAT probability of biodegradation	1
EPI Predicted hydrolysis half-life (days)	1.28E+003
EPI Predicted Ozone reaction half-life (days)	999
Experimental Atmospheric Oxidation half-life (days)	2.16486729644624
Reference (oxidation)	Atkinson R; Kinetics and mechanisms of the gas-phase reactions of the hydroxyl radical with organic compounds.; Journal of Physical and Chemical Reference Data. Monograph No. 1.; 1989
EPI Predicted Atmospheric Oxidation half-life (days)	3.208

ROBUST STUDY SUMMARY - Persistence

Item	Yes	No
Reference: Atkinson, R. 1989. Kinetics and Mechanisms of the Gas-Phase Reactions of the Hydroxyl Radical with Organic Compounds. Journal of Physical and Chemical Reference Data, Monograph No. 1.		
Test Substance (CAS # and name): 105-54-4 (Ethyl butyrate)		
Chemical composition of the substance (including purity, by-products)		X
Method		
References	X	
OECD, EU, national, or other standard method?		X
Justification of the method/protocol if not a standard method was used		
Test design / conditions		
Study type (photodegradation, hydrolysis, biodegradation, other –specify, do not assess): -OH radical reaction		
Test type (aerobic or anaerobic - specify, do not assess): Aerobic		

Test medium (air, water, soil, sediment - specify, do not assess): Air		
Information on stability of the substance in the media of concern is reported?		X
Controls (positive or negative): Not mentioned		X
Number of replicates (including controls)		X
Temperature	X	
Duration of the experiment		X
For photodegradation only		
Light source (specify):		
Light spectrum and relative intensity based on sunlight intensity:		
For hydrolysis only		
Measured concentrations reported?		
Basic water properties (pH, hardness, etc.)		
For biodegradation only		
Ready or inherent biodegradation (specify):		
Inoculum (concentration and source):		
Results		
Endpoints: half-life (preferred); degradation, %; etc. (do not assess this item): 1.1 d (half-life); 4.94 x 10-12 cm3 molecule-1 second-1		
Information on breakdown products (do not assess this item): No		
Overall score: 2 /8 = 25%		
EC Reliability code: 2		
Reliability category (high, satisfactory, low): Satisfactory with restrictions		
Comments:Score = 25%; Chemical purity – not mentioned. No standard methods mentioned in review. Temperature = 23°C. This value is slightly different than the one in the EC database. Half-life determined from OH concentration = 1.5E6 molecules/cm^(3). Reaction rate is suggested by Atkinson from T.J. Wallington, P Dagaut, R. Liu and M.J. Kurylo. 1988. Int. J. Chem. Kinet. 20, p. 177. This article has been suggested for further review. Satisfactory confidence with restrictions; reliability code = 2.		

Data accessed May 2017 on the OECD website: http://webnet.oecd.org/CCRWeb/Search.aspx

EPISuite provides the following information:

Henrys Law Constant (25 deg C) [HENRYWIN v3.20]:

Bond Method:	4.10E-004 atm-m3/mole (4.16E+001 Pa-m3/mole)
Group Method:	3.15E-004 atm-m3/mole (3.19E+001 Pa-m3/mole)
Exper Database:	3.99E-04 atm-m3/mole (4.04E+001 Pa-m3/mole)
Henrys LC [via VP/WSol estimate using User-Entered or Estimated values]:	HLC: 8.129E-004 atm-m3/mole (8.237E+001 Pa-m3/mole) VP: 14.6 mm Hg (source: MPBPVP) WS: 2.75E+003 mg/L (source: WSKOWWIN)

Log Octanol-Air Partition Coefficient (25 deg C) [KOAWIN v1.10]:

Log Kow used:	1.85 (KowWin est)
Log Kaw used:	-1.787 (exp database)
Log Koa (KOAWIN v1.10 estimate):	3.637
Log Koa (experimental database):	3.560

Probability of Rapid Biodegradation (BIOWIN v4.10):

Biowin1 (Linear Model): Biowin2 (Non-Linear Model) : Biowin3 (Ultimate Survey Model): Biowin4 (Primary Survey Model) : Biowin5 (MITI Linear Model) : Biowin6 (MITI Non-Linear Model): Biowin7 (Anaerobic Linear Model):	0.8664 0.9957 3.0827 (weeks) 3.9091 (days) 0.8594 0.9501 0.9268
Ready Biodegradability Prediction:	YES

Hydrocarbon Biodegradation (BioHCwin v1.01):

Structure incompatible with current estimation method!

Sorption to aerosols (25 Dec C)[AEROWIN v1.00]:

Vapor pressure (liquid/subcooled):	1.71E+003 Pa (12.8 mm Hg)
Log Koa (Koawin est):	3.560
Kp (particle/gas partition coef. (m3/ug)): Mackay model: Octanol/air (Koa) model:	1.76E-009 8.91E-010

Fraction sorbed to airborne particulates (phi):

Junge-Pankow model:	6.35E-008
Mackay model:	1.41E-007
Octanol/air (Koa) model:	7.13E-008

Atmospheric Oxidation (25 deg C) [AopWin v1.92]: Hydroxyl Radicals Reaction:

OVERALL OH Rate Constant =	3.3339 E-12 cm3/molecule-sec
Half-Life =	3.208 Days (12-hr day; 1.5E6 OH/cm3)
Half-Life =	38.499 Hrs
Ozone Reaction:	No Ozone Reaction Estimation
Fraction sorbed to airborne particulates (phi): 1.02E-007 (Junge-Pankow, Mackay avg) 7.13E-008 (Koa method) Note: the sorbed fraction may be resistant to atmospheric oxidation	

Soil Adsorption Coefficient (KOCWIN v2.00):

Koc :	19.41 L/kg (MCI method)
Log Koc:	1.288 (MCI method)
Koc :	76.35 L/kg (Kow method)
Log Koc:	1.883 (Kow method)

Aqueous Base/Acid-Catalyzed Hydrolysis (25 deg C) [HYDROWIN v2.00]:

Total Kb for pH > 8 at 25 deg C:	6.266E-002 L/mol-sec
Kb Half-Life at pH 8:	128.021 days
Kb Half-Life at pH 7:	3.505 years

(Total Kb applies only to esters, carbmates, alkyl halides)
Volatilization from Water: Henry LC: 0.000399 atm-m3/mole (Henry experimental database)

Half-Life from Model River:	2.681 hours
Half-Life from Model Lake:	119.6 hours (4.984 days)

Removal In Wastewater Treatment:

Total removal:	17.07 percent

Total biodegradation:	0.08 percent
Total sludge adsorption:	1.78 percent
Total to Air:	15.21 percent

(using 10000 hr Bio P,A,S)
Level III Fugacity Model:

	Mass Amount (percent)	Half-Life (hr)	Emissions (kg/hr)
13.7	13.7	52	1000
39.8	39.8	360	1000
46.4	46.4	720	1000
0.105	0.105	3.24e+003	0

Persistence Time: 208 hr

10.2. Aquatic toxicity

The Ecological Categorization Results from the Canadian Domestic Substances List state that ethyl butyrate is not inherently toxic to aquatic organisms:

Pivotal value for iT (mg/l)	750
Experimental result iT (mg/l)	750
Test species iT (Latin)	Daphnia mag
Test species iT (Common)	Water flea
Final EndPoint iT	EC50
Exposure duration iT (hours)	24
Reference iT	Z.Wasser-Abwasser-Forsch. 15(1):1-6 (GER) (ENG ABS) (OECDG Data File)
Toxicity to fathead minnow (LC50 in mg/l) as predicted by Topkat v6.1	101.4
Toxicity to fish (LC50 in mg/l) as predicted by Ecosar v0.99g	21.15
Toxicity to fish (LC50 in mg/l) as predicted by Oasis Forecast M v1.10	117.8795
Toxicity to fish (LC50 in mg/l) as predicted by Aster	23.742635
Toxicity to fish (LC50 in mg/l) as predicted by PNN	21.42801
Toxicity to fish, daphnia, algae or mysid shrimp (EC50 or LC50 in mg/l) as predicted by Ecosar v0.99g	126.346

Toxicity to fish (LC50 in mg/l) as predicted by Neutral Organics QSAR in Ecosar v0.99g	3.97E+001

ROBUST STUDY SUMMARY - Inherent Toxicity

Item	Yes	No
Reference: Bringmann G and R. Kühn. 1982. Ergebnisse der Schadwirkung wassergefährdender Stoffe gegen Daphnia magna in einem weiterentwickelten standardisierten Testverfahren. Z. Abwasser Forsch. 15(1982) 1:1-6.		
Test Substance: CAS # and name: CAS # 105-54-4; Butanoic acid, Ethyl ester		
*Chemical composition of the substance (including purity, by-products)		x
Persistence/stability of test substance in aquatic solution		x
Method		
References (More details on test).	x	
*OECD, EU, national, or other standard method? Standardised Daphnia toxicity test.	x	
Justification of the method/protocol if not a standard method was used	NA	
*GLP (Good Laboratory Practice) Not applicable.	NA	
Test organisms (specify common and Latin names) Daphnia magna – water flea.		
Latin or both Latin and common names reported?	x	
Life cycle age / stage of test organism Not applicable.	NA	
Sex. Not applicable.	NA	
Length and weight of test organisms. Max. 24 hours old. Not applicable.	NA	
Number of test organisms per replicate. Ten test organisms.	x	
Food type / feeding periods (acclimation/during test) .	x	
Test design / conditions		
Test type – acute or chronic (specify, but do not assess this item): acute 24 h EC_{50}		
Experiment type (laboratory or field) specified?	x	
System type (static, semi-static, flow-through)?	x	
Negative or positive controls (specify)?		x
Number of replicates (including controls) and concentrations 3 replicates.	x	
Exposure pathways (food, water, both)		x

Exposure duration	x	
*Measured concentrations reported? Nominal conc.		x
Exposure media conditions (temperature, pH, electrical conductivity, hardness, TOC, DOC, DO, major cations and anions; other)	x	
Was pH within 6-9 range? (do not assess this item)	x	
Was temperature within 5-28 °C range? (do not assess this item)	x	
Photoperiod and light intensity	x	
Stock and test solution preparation Dilution series.	x	
Use of emulgators / solubilizers (especially for poorly soluble / unstable substances) Not used.	NA	
Analytical monitoring intervals		x
Statistical methods used: Regression.	x	
Results		
Toxicity values (LC50, EC50, or IC50 - EC50 = 750mg/L		
Other endpoints reported - BCF/BAF, LOEC/NOEC (specify, do not assess this item): No		
*Was toxicity value below the chemical's water solubility?	x	
Other adverse effects (carcinogenicity, mutagenicity, etc. Do not assess this item) No.		
Score: major items – 2/4, 16/22= 73%		
EC Reliability code: 2		
Reliability category (high, satisfactory, low): Satisfactory		
Comments:		

Data accessed May 2017 on the OECD website: http://webnet.oecd.org/CCRWeb/Search.aspx

Record for butanoic acid, ethyl ester (CAS RN 105-54-4):

Spec. Sci. Name **Spec. Common Name**	**Exposure Type**	**Media Type**	**Test Location**	**Obs. Dur. (Days)**	**Endpoint**	**Effect**	**Effect Meas.**	**Conc. Type Conc. (Std)**	**Chem. Anal.**
Chlorococcales Green Algae Order	S	FW		1 d	EC10	PHY	ASML	F 340000 ug/L	
Chlorococcales Green Algae	S	FW		1 d	EC50	PHY	ASML	F 1000000	

Order								ug/L	
Scenedesmus quadricauda Green Algae	S	FW	LAB		LOEC	POP	PGRT/	F 47000 ug/L	U
Microcystis aeruginosa Blue-Green Algae	S	FW	LAB		LOEC	POP	PGRT/	F 700000 ug/L	U
Entosiphon sulcatum Flagellate Euglenoid	S	FW	LAB	3 d		POP	ABND	F 236000 ug/L	U
Scenedesmus quadricauda Green Algae	S	FW	LAB	7 d		POP	ABND	F 47000 ug/L	U
Entosiphon sulcatum Flagellate Euglenoid	S	FW	LAB	3 d		POP	PGRT/	F 236000 ug/L	U
Entosiphon sulcatum Flagellate Euglenoid	AQUA - NR	FW	LAB			POP	PGRT/	F 236000 ug/L	
Microcystis aeruginosa Blue-Green Algae	AQUA - NR	FW	LAB	8 d		POP	GPOP/	F 700000 ug/L	U
Scenedesmus quadricauda Green Algae	AQUA - NR	FW	LAB			POP	PGRT/	F 47000 ug/L	
Chilomonas paramecium Cryptomonad	AQUA - NR	FW	LAB			POP	PGRT/	F > 2480000 ug/L	
Chilomonas paramecium Cryptomonad	AQUA - NR		LAB	2 d		POP/	GPOP	F > 2480000 ug/L	U
Anacystis aeruginosa	S		LAB	8 d		POP/	GPOP	F 700000	U

Blue-Green Algae								ug/L	
Scenedesmus quadricauda Green Algae	AQUA - NR	FW	LAB	10 d		POP	BMAS/	F 47000 ug/L	U
Scenedesmus quadricauda Green Algae	S		LAB	8 d		POP/	GPOP	F 47000 ug/L	U
Entosiphon sulcatum Flagellate Euglenoid	AQUA - NR	FW	LAB			POP	PGRT/	F 236000 ug/L	
Daphnia magna Water Flea	AQUA - NR	FW		1 d	EC0	BEH	EQUL	F 155000 ug/L	
Daphnia magna Water Flea	AQUA - NR	FW		1 d	EC100	BEH	EQUL	F 3200000 ug/L	
Daphnia magna Water Flea	AQUA - NR	FW		1 d	EC50	BEH	EQUL	F 750000 ug/L	
Daphnia magna Water Flea	S	FW	LAB	1 d	LC0	ITX	IMBL	F 47000 ug/L	U
Daphnia magna Water Flea	S	FW	LAB	1 d	LC100	ITX	IMBL	F 1155000 ug/L	U
Daphnia magna Water Flea	S	FW	LAB	1 d	LC50	ITX	IMBL	F 755000 ug/L	U
Leuciscus idus ssp. melanotus Carp	AQUA - NR	FW		2 d	LC0	MOR	MORT	F 35000 ug/L	
Leuciscus idus ssp. melanotus	AQUA - NR	FW		2 d	LC0	MOR	MORT	F 61000 ug/L	

Carp									
Leuciscus idus ssp. melanotus Carp	AQUA - NR	FW		2 d	LC100	MOR	MORT	F 87000 ug/L	
Leuciscus idus ssp. melanotus Carp	AQUA - NR	FW		2 d	LC100	MOR	MORT	F 131000 ug/L	
Leuciscus idus ssp. melanotus Carp	AQUA - NR	FW		2 d	LC50	MOR	MORT	F 53000 ug/L	
Leuciscus idus ssp. melanotus Carp	AQUA - NR	FW		2 d	LC50	MOR	MORT	F 78000 ug/L	
Uronema parduczi Ciliate	AQUA - NR	FW	LAB			POP	PGRT/	F 916000 ug/L	
Uronema parduczi Ciliate	AQUA - NR	FW	LAB	.8333 d		POP	PGRT/	F 916000 ug/L	

As taken from the EPA ECOTOX Database, accessed May 2017, available at http://cfpub.epa.gov/ecotox/advanced_query.htm

ECOSAR Version 1.11 reports the following aquatic toxicity data for CAS RN 105-54-4:

Values used to Generate ECOSAR Profile
Log Kow: 1.846 (EPISuite Kowwin v1.68 Estimate)
Wat Sol: 4900 (mg/L, PhysProp DB exp value)

ECOSAR v1.11 Class(es) found:
Esters

ECOSAR Class	Organism	Duration	End Pt	Predicted mg/L (ppm)	

Esters :	Fish	96-hr	LC50	19.093	
Esters :	Daphnid	48-hr	LC50	40.347	
Esters :	Green Algae	96-hr	EC50	17.594	
Esters :	Fish		ChV	1.483	
Esters :	Daphnid		ChV	28.833	
Esters :	Green Algae		ChV	4.291	
Esters :	Fish (SW)	96-hr	LC50	29.319	
Esters :	Mysid	96-hr	LC50	31.104	
Esters :	Fish (SW)		ChV	4.088	
Esters :	Mysid (SW)		ChV	3130.196	
Neutral OrganicSAR :	Fish	96-hr	LC50	131.102	
(BaselineToxicity) :	Daphnid	48-hr	LC50	73.392	
	Green Algae	96-hr	EC50	51.557	
	Fish		ChV	12.602	
	Daphnid		ChV	6.880	
	Green Algae		ChV	13.082	

EC50; Species: Chlorococcales (Green Algae Order); Conditions: freshwater, static; Concentration: 1000000 ug/L for 24 hr; Effect: physiology, assimilation efficiency /formulated product/ [Krebs F; Dtsch Gewaesserkd Mitt 35 (5-6): 161-170 (1991) as cited in the ECOTOX database. Available from, as of April 26, 2015: http://cfpub.epa.gov/ecotox/ **PEER REVIEWED**

LC50; Species: Daphnia magna (Water Flea) age < or =24 hr; Conditions: freshwater, static, 20-22 deg C, pH 7.6-7.7; Concentration: 755000 ug/L for 24 hr /formulated product/ [Bringmann G, Kuhn R; Z Wasser-Abwasser-Forsch 10 (5): 161-166 (1977) as cited in the ECOTOX database. Available from, as of April 26, 2015: http://cfpub.epa.gov/ecotox/ **PEER REVIEWED**

As taken from HSDB, 2015.

10.3. Sediment toxicity

No data available to us at this time.

10.4. Terrestrial toxicity

Record for butanoic acid, ethyl ester (CAS RN 105-54-4)

Spec. Sci.	Media	Test Loc	Exp. Typ	Dose	Endpoin	Effec	Effect	Resp	Chem	Conc.	Obs. Dur.

Name Spec. Common Name	Type	.	e	#	t	t	Meas.	. Site	. Anal.	Type Dose	(Days)
Solanum tuberosum Potato	CUL	LAB	SO			INJ	DAMG	TU		NC (0.03-0.04) M	1 d
Boiga irregularis Brown Tree Snake	NONE	LAB	HS	1	NOEL	BEH	NMVM		U	A 1/ (NR/-NR/) % w/w	0.004 d

As taken from the EPA ECOTOX database, accessed May 2017, available at http://cfpub.epa.gov/ecotox/advanced_query.htm

ECOSAR Version 1.11 reports the following terrestrial toxicity data:

Values used to Generate ECOSAR Profile
Log Kow: 1.846 (EPISuite Kowwin v1.68 Estimate)
Wat Sol: 4900 (mg/L, PhysProp DB exp value)

ECOSAR v1.11 Class(es) found:
Esters

ECOSAR Class	Organism	Duration	End Pt	Predicted mg/L (ppm)
Esters :	Earthworm	14-day	LC50	1697.393

10.5. All other relevant types of ecotoxicity

EPISuite provides the following information:
Bioaccumulation Estimates (BCFBAF v3.01):

Log BCF from regression-based method:	0.885 (BCF = 7.678 L/kg wet-wt)
Log Biotransformation Half-life (HL):	-1.4652 days (HL = 0.03426 days)
Log BCF Arnot-Gobas method (upper trophic):	0.628 (BCF = 4.243)
Log BAF Arnot-Gobas method (upper trophic):	0.628 (BAF = 4.243)
log Kow used:	1.85 (estimated)

The Ecological Categorization Results from the Canadian Domestic Substances List state that ethyl butyrate is not bioaccumulative in the environment:

Log Kow predicted by KowWin	1.85
Log BAF T2MTL predicted by Gobas	0.733349646445362
Log BCF 5% T2LTL predicted by Gobas	0.65888545104743
Log BCF Max predicted by OASIS	1.6086360923561
Log BCF predicted by BCFWIN	0.722

Data accessed May 2017 on the OECD website: http://webnet.oecd.org/CCRWeb/Search.aspx

11. References

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13. Last audited

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TOXICOLOGICAL EVALUATION OF SOME FLAVOURING SUBSTANCES AND NON-NUTRITIVE SWEETENING AGENTS

Geneva, 21-28 August 1967

The Eleventh Report of the Joint FAO/WHO Expert Committee on Food Additives is published as FAO Nutrition Meetings Report Series, 1967, No. 44; Wld Hlth Org. techn. Rep. Ser., 1968, 383. This Report contains general considerations, including the principles adopted for the evaluation, and a summary of the results of the evaluations of a number of food additives. Additional information, such as biological data and a toxicological evaluation, considered at that meeting, is to be found in this document.

Food and Agriculture Organization of the United Nations
World Health Organization
1967

ETHYL BUTYRATE

Chemical name	Ethyl butanoate
Empirical formula	$C_6H_{12}O_2$
Structural formula	$CH_3CH_2CH_2COOC_2H_5$
Molecular weight	116.16
Definition	Ethyl butyrate contains not less than 97

per cent. $C_6H_{12}O_2$.

Description — Ethyl butyrate may be prepared by the esterification of normal butyric acid with alcohol. It is a colourless liquid with a fruity odour

Biological data

Biolochemical aspects

This ester is hydrolysed by pancreatic lipase to ethyl alcohol and butyric acid (Oppenheimer, 1925; Nishida, 1957).

Acute toxicity

Animal	Route	LD_{50} (mg/kg body-weight)	References
Rat	oral	13 050	Jenner et al., 1964

Short-term studies

Rat. In a feeding experiment on 15 males and 15 females for 12 weeks no adverse effect was noted at 14.4 mg/kg body-weight/day (Oser, 1967).

Long-term studies

None available.

Comments

This compound hydrolyzes into normal constituents of food. Evaluation is based on this and the short-term studies. Further biochemical and metabolic studies are considered desirable.

EVALUATION

Estimate of acceptable daily intake for man

mg/kg body-weight

Unconditional acceptance 0-15

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See Also:

Toxicological Abbreviations

ETHYL BUTYRATE (JECFA Evaluation)

Summary of Evaluations Performed by the Joint FAO/WHO Expert Committee on Food Additives

ETHYL BUTYRATE

COE No.:	264
FEMA No.:	2427
JECFA No.:	29
Chemical names:	ETHYL BUTANOATE
Functional class:	FLAVOURING AGENT
Latest evaluation:	1996
ADI:	0-15 mg/kg bw (1967)
Comments:	No safety concern at current levels of intake when used as a flavouring agent. The 1967 ADI of 0-15 mg/kg bw was maintained at the forty-sixth meeting (1996).
Report:	TRS 868-JECFA 46/21
Specifications:	COMPENDIUM ADDENDUM 4/FNP 52 Add.4/177
Tox monograph:	See TRS 868-JECFA 46/64
Previous status:	1967, NMRS 44/TRS 383-JECFA 11/12, FAS 69.31/NMRS 44B-JECFA 11/17 (COMPENDIUM/587), FAS 68.33/NMRS 44A-JECFA 11/25. 0-15. FU. N

30 Apr 02

See Also:
Toxicological Abbreviations
Ethyl butyrate (FAO Nutrition Meetings Report Series 44a)

Inhalation Toxicology, 18:685–706, 2006

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Effects of Flavoring and Casing Ingredients on the Toxicity of Mainstream Cigarette Smoke in Rats

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A series of in vitro and in vivo studies evaluated the potential effects of tobacco flavoring and casing ingredients. Study 1 utilized as a reference control cigarette a typical commercial tobacco blend without flavoring ingredients, and a test cigarette containing a mixture of 165 low-use flavoring ingredients. Study 2 utilized the same reference control cigarette as used in study 1 and a test cigarette containing eight high-use ingredients. The in vitro Ames *Salmonella typhimurium* assay did not show any increase in mutagenicity of smoke condensate from test cigarettes designed for studies 1 and 2 as compared to the reference. Sprague-Dawley rats were exposed by nose-only inhalation for 1 h/day, 5 days/wk for 13 wk to smoke from the test or reference cigarettes already described, or to air only, and necropsied after 13 wk of exposure or following 13 wk of recovery from smoke exposure. Exposure to smoke from reference or test cigarettes in both studies induced increases in blood carboxyhemoglobin (COHb) and plasma nicotine, decreases in minute volume, differences in body or organ weights compared to air controls, and a concentration-related hyperplasia, squamous metaplasia, and inflammation in the respiratory tract. All these effects were greatly decreased or absent following the recovery period. Comparison of rats exposed to similar concentrations of test and reference cigarette smoke indicated no difference at any concentration. In summary, the results did not indicate any consistent differences in toxicologic effects between smoke from cigarettes containing the flavoring or casing ingredients and reference cigarettes.

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Flavoring ingredients are added to tobacco during the manufacture of many types of commercial cigarettes, and humectants such as glycerol are added to increase the moisture-holding capacity of the tobacco. There has been much speculation about the effect of these added ingredients on the toxicity of the resultant smoke. Wynder and Hoffman (1967) hypothesized that adding

nontobacco ingredients might increase or decrease the toxic effects of inhaled tobacco smoke, and later publications (LaVoie et al., 1980; Hoffman and Hoffman, 1997, 2001; World Health Organization, 2001) supported that hypothesis. Recently published research results (Gaworski et al., 1998; Paschke et al., 2002; Rodgman, 2002a, 2002b; Rodgman and Green, 2002; Carmines, 2002; Rustemeier et al., 2002; Roemer et al., 2002; Vanscheeuwijck et al., 2002; Baker et al., 2004) have presented data from in vitro, and in vivo toxicity studies that indicate the addition of ingredients to tobacco does not increase the toxicity of the smoke. Baker et al. (2004), using a pyrolysis technique that mimics closely the combustion conditions inside burning cigarettes (Baker and Bishop, 2004), studied the effects of pyrolysis on the chemistry, in vitro genotoxicity and cytotoxicity, and inhalation toxicity in rodents of 291 single ingredients added to cigarettes.

The studies described herein were designed to evaluate the potential influence of low-use flavoring ingredients and high-use mixed casing or flavoring ingredients on the biological activity of mainstream cigarette smoke. Test cigarettes containing flavorings or casings were analyzed and compared against an identical reference cigarette respectively produced without flavors or casings.

MATERIALS AND METHODS

Cigarette Design

In study 1, 165 low-use flavoring ingredients were added to a single test cigarette and compared to a reference cigarette without these ingredients. In study 2, eight high-use flavoring or casing ingredients were added to a single test cigarette and compared to the same reference cigarette that was used in study 1. Thus, the design covered these ingredients as well as possible interactions between them and/or their combustion or pyrolysis products. The prototype cigarettes were designed to be representative of commercial, full flavor filter cigarettes. Test and reference cigarettes were constructed with conventional commercial equipment.

The ingredients selected for evaluation in these studies comprise low-use and high-use ingredients normally utilized in the manufacture of commercial cigarettes. The point of addition was chosen to mimic actual process conditions. Study 1 and study 2 ingredients were incorporated into a flavoring or casing system at levels exceeding their normal use. Table 1 outlines the tobacco components of the blend used to construct the cigarettes in both study 1 and study 2. The blends were cased with a mixture of glycerin and water (at a ratio of 2:1) to provide the necessary moisture for standard processing. In preparation of study 1 cigarettes, the ingredients were applied at a rate of 10 kg/1000 kg leaf blend, that is, at 1% on the test cigarettes, and the casing was applied at a rate of 30 kg/1000 kg leaf blend. The study 2 ingredient system was applied at a rate of 31 kg/1000 kg leaf blend (3.1%). The 165 ingredients included in the study 1 mixture appear listed in order of descending application rate in Table 2,

TABLE 1
Blend composition of prototype cigarettes

	Percent of blend component in cigarettes	
Blend components	Tobacco wet weight	Tobacco dry weight
Burley	24	22.9
Virginia	28	25.7
Oriental	14.8	13.6
Reconstituted sheet	23.4	20.1
Expanded tobacco	9.7	8.8

along with the corresponding CAS-Number, regulatory identifiers (where applicable) and application rate. The seven casings and one flavoring included in the study 2 mixture appear listed in order of descending application rate in Table 3. Cellulose acetate filters with 32% average air dilution were used in all cigarettes. Monogram inks were not subject to these studies.

Cigarette Performance

A preliminary cigarette performance evaluation was carried out prior to the toxicology studies. Prior to characterization, the cigarettes were conditioned for a minimum of 48 h at a temperature of $22 \pm 1°C$ and a relative humidity (RH) of $60 \pm 2\%$, in accordance with ISO Standard 3402. Subsequently, the cigarettes were smoked on a 20-port Borgwaldt smoking machine under the conditions stipulated in ISO Standard 3308. Therefore, the puffing regime for mainstream smoke used a 35 ± 0.3 ml puff volume, with 2.0 ± 0.05 s puff duration once every 60 ± 0.5 s. Smoke samples were respectively collected in accordance with the analytical method.

In Vitro Study Design

The mutagenicity of total particulate matter (TPM) in study 1 and 2 cigarettes was investigated using an Ames assay protocol that conformed to OECD Guideline 471. For this purpose, prototype cigarettes containing a mixture of ingredients, reference cigarettes without these ingredients, and 2R4F cigarettes (a standard reference cigarette developed and validated by the University of Kentucky) were smoked on a Borgwaldt RM200 rotary smoking machine under the ISO standard 3308 condition. TPM was collected in a standard fiberglass (Cambridge) trap with dimethyl sulfoxide (DMSO), and the DMSO solution was stored in the dark at $-80°C$ prior to performance of the Ames assay. Each sample was tested with and without S9 metabolic activation in five strains of *Salmonella typhimurium*: TA98, TA100, TA102, TA1535, and TA1537. Evaluation of the Ames assay data was carried out in terms of the mutagenic response, taking into consideration the reproducibly dose-related increase in number of revertants, even if the increase was less than twofold. The mutagenic response to TPM from the reference and test cigarettes was compared using the linear portion of the slope (revertants/mg TPM).

TABLE 2
Ingredients added to test cigarettes in study 1

	Ingredient	CAS no.[a]	FEMA no.[b]	CFR[c]	CoE[d]	Application rate (ppm)
1	Benzyl alcohol	100-51-6	2137	172.515	58c	260
2	Immortelle extract	8023-95-8	2592	182.20	225n	156
3	Coriander oil	8008-52-4	2334	182.20	154n	65
4	Balsam peru resinoid	8007-00-9	2117	182.20	298n	65
5	Anise star oil	8007-70-3	2096	N.A.	238n	65
6	Celery seed oil	89997-35-3	2271	182.20	52n	65
7	Vanillin	121-33-5	3107	182.60	107c	65
8	Potassium sorbate	24634-61-5	2921	182.3640	N.A.	39
9	Propyl *para*-hydroxybenzoate	94-13-3	2951	172.515	N.A.	39
10	Benzoin resinoid	9000-05-9	2133	172.510	439n	26
11	Cedarwood oil	8000-27-9	N.A.	N.A.	252n	26
12	Clary extract	8016-63-5	2321	182.20	415n	26
13	Methylcyclopentenolone	80-71-7	2700	172.515	758c	26
14	Phenethyl alcohol	60-12-8	2858	172.515	68c	26
15	Piperonal	120-57-0	2911	182.60	104c	26
16	Tea extract	84650-60-2	N.A.	182.20	451n	26
17	Vanilla oleoresin	8024-06-4	3106	182.20	474n	26
18	Brandy	N.A.	N.A.	N.A.	N.A.	26
19	*trans*-Anethole	4180-23-8	2086	182.60	183c	19.5
20	Coffee extract	84650-00-0	N.A.	182.20	452n	19.5
21	5-Ethyl-3-hydroxy-4-methyl-2(5*H*)-furanone	698-10-2	3153	N.A.	2300c	19.5
22	Propionic acid	79-09-4	2924	184.1081	3c	13
23	Acetic acid	64-19-7	2006	184.1005	2c	13
24	Amyl formate	638-49-3	2068	172.515	497c	13
25	Angelica root oil	8015-64-3	2088	182.20	56n	13
26	Beeswax absolute	8012-89-3	2126	184.1973	N.A.	13
27	Benzyl benzoate	120-51-4	2138	172.515	262c	13
28	Benzyl propionate	122-63-4	2150	172.515	413c	13
29	Cardamom oil	8000-66-6	2241	182.20	180n	13
30	beta-Carotene	7235-40-7	N.A.	184.1245	N.A.	13
31	Ethyl acetate	141-78-6	2414	182.60	191c	13
32	Ethyl butyrate	105-54-4	2427	182.60	264c	13
33	Ethyl levulinate	539-88-8	2442	172.515	373c	13
34	Eucalyptol	470-82-6	2465	172.515	182c	13
35	Geranium oil	8000-46-2	2508	182.20	324n	13
36	Labdanum resinoid	8016-26-0	2610	172.510	134n	13
37	Lavandin oil	8022-15-9	2618	182.20	257n	13
38	Maltol	118-71-8	2656	172.515	148c	13
39	Spearmint oil	8008-79-5	3032	182.20	285n	13
40	Ethyl hexanoate	123-66-0	2439	172.515	310c	10.4
41	Acetylpyrazine	22047-25-2	3126	N.A.	2286c	9.1
42	Ethylmaltol	4940-11-8	3487	172.515	692c	9.1
43	Chamomile oil, Roman	8015-92-7	2275	182.20	48n	6.5
44	Citronella oil	8000-29-1	2308	182.20	39n	6.5
45	delta-Decalactone	705-86-2	2361	172.515	621c	6.5
46	gamma-Decalactone	706-14-9	2360	172.515	2230c	6.5
47	Ethyl phenylacetate	101-97-3	2452	172.515	2156c	6.5

(Continued on next page)

TABLE 2
Ingredients added to test cigarettes in study 1 *(Continued)*

	Ingredient	CAS no.[a]	FEMA no.[b]	CFR[c]	CoE[d]	Application rate (ppm)
48	Ethyl valerate	539-82-2	2462	172.515	465c	6.5
49	Ethyl vanillin	121-32-4	2464	182.60	108c	6.5
50	Fennel sweet oil	8006-84-6	2485	182.20	200n	6.5
51	Glycyrrhizin ammoniated	53956-04-0	N.A.	184.1408	N.A.	6.5
52	gamma-Heptalactone	105-21-5	2539	172.515	2253c	6.5
53	3-Hexen-1-ol	928-96-1	2563	172.515	750c	6.5
54	3-Hexenoic acid	1577-18-0	3170	N.A.	2256c	6.5
55	Hexyl alcohol	111-27-3	2567	172.515	53c	6.5
56	Isoamyl phenylacetate	102-19-2	2081	172.515	2161c	6.5
57	Methyl phenylacetate	101-41-7	2733	172.515	2155c	6.5
58	Nerol	106-25-2	2770	172.515	2018c	6.5
59	Nerolidol	142-50-7	2272	172.515	67c	6.5
60	Peruvian (bois de rose) oil	8015-77-8	2156	182.20	44n	6.5
61	Phenylacetic acid	103-82-2	2878	172.515	672c	6.5
62	Pyruvic acid	127-17-3	2970	172.515	19c	6.5
63	Rose absolute	8007-01-0	2988	182.20	405n	6.5
64	Sandalwood oil	8006-87-9	3005	172.510	420n	6.5
65	Sclareolide	564-20-5	3794	N.A.	N.A.	6.5
66	Triethyl citrate	77-93-0	3083	184.1911	N.A.	6.5
67	2,3 5-Trimethylpyrazine	14667-55-1	3244	N.A.	735c	6.5
68	Olibanum absolute	8016-36-2	2816	172.510	93n	6.5
69	delta-Octalactone	698-76-0	3214	N.A.	2195c	6.5
70	2-Hexenal	6728-26-3	2560	172.515	748c	5.2
71	Ethyl octadecanoate	111-61-5	3490	N.A.	N.A.	5.2
72	4-Hydroxy-3-pentenoic acid lactone	591-12-8	3293	N.A.	731c	3.9
73	Methyl 2-pyrrolyl ketone	1072-83-9	3202	N.A.	N.A.	3.9
74	Methyl linoleate (48%) methyl linolenate (52%) mixture	112-63-0 301-00-8	3411	N.A.	713c	3.9
75	Petitgrain mandarin oil	8014-17-3	2854	182.20	142n	3.9
76	Propenylguaethol	94-86-0	2922	172.515	170c	3.9
77	4-(2,6,6-Trimethylcyclohexa-1,3-dienyl) but-2-en-4-one	23696-85-7	3420	N.A.	N.A.	3.9
78	2-Propionyl pyrrole	1073-26-3	3614	N.A.	N.A.	3.9
79	Orange essence oil	8008-57-9	2825	182.20	143n	2.6
80	Benzyl phenylacetate	102-16-9	2419	172.515	232c	2.6
81	2,3-Butanedione	431-03-8	2370	184.1278	752c	1.95
82	2,3,5,6-Tetramethylpyrazine	1124-11-4	3237	N.A.	734c	1.95
83	Hexanoic acid	142-62-1	2559	172.515	9c	1.56
84	Cinnamaldehyde	104-55-2	2286	182.60	102c	1.3
85	Acetophenone	98-86-2	2009	172.515	138c	1.3
86	2-Acetylthiazole	24295-03-2	3328	N.A.	N.A.	1.3
87	Amyl alcohol	71-41-0	2056	172.515	514c	1.3
88	Amyl butyrate	540-18-1	2059	172.515	270c	1.3
89	Benzaldehyde	100-52-7	2127	182.60	101c	1.3
90	Butyl butyrate	109-21-7	2186	172.515	268c	1.3
91	Butyric acid	107-92-6	2221	182.60	5c	1.3
92	Cinnamyl alcohol	104-54-1	2294	172.515	65c	1.3

(Continued on next page)

TABLE 2
Ingredients added to test cigarettes in study 1 *(Continued)*

	Ingredient	CAS no.[a]	FEMA no.[b]	CFR[c]	CoE[d]	Application rate (ppm)
93	DL-Citronellol	106-22-9	2309	172.515	59c	1.3
94	Decanoic acid	334-48-5	2364	172.860	11c	1.3
95	para-Dimethoxybenzene	150-78-7	2386	172.515	2059c	1.3
96	3,4-Dimethyl-1,2-cyclopentanedione	13494-06-9	3268	N.A.	2234c	1.3
97	Ethylbenzoate	93-89-0	2422	172.515	261c	1.3
98	Ethyl heptanoate	106-30-9	2437	172.515	365c	1.3
99	Ethyl isovalerate	108-64-5	2463	172.515	442c	1.3
100	Ethyl myristate	124-06-1	2445	172.515	385c	1.3
101	Ethyl octanoate	106-32-1	2449	172.515	392c	1.3
102	Ethyl palmitate	628-97-7	2451	N.A.	634c	1.3
103	Ethyl propionate	105-37-3	2456	172.515	402c	1.3
104	2-Ethyl-3-methylpyrazine	15707-23-0	3155	N.A.	548c	1.3
105	Genet absolute	8023-80-1	2504	172.510	436n	1.3
106	Geraniol	106-24-1	2507	182.60	60c	1.3
107	Geranyl acetate	105-87-3	2509	182.60	201c	1.3
108	gamma-Hexalactone	695-06-7	2556	172.515	2254c	1.3
109	Hexyl acetate	142-92-7	2565	172.515	196c	1.3
110	Isoamyl acetate	123-92-2	2055	172.515	214c	1.3
111	Isoamyl butyrate	106-27-4	2060	172.515	282c	1.3
112	3,7-Dimethyl-1,6-octadiene-3-ol	78-70-6	2635	182.60	61c	1.3
113	Menthyl acetate	89-48-5	2668	172.515	206c	1.3
114	Methyl isovalerate	556-24-1	2753	172.515	457c	1.3
115	Methyl salicylate	119-36-8	2745	175.105	433c	1.3
116	3-Methylpentanoic acid	105-43-1	3437	N.A.	N.A.	1.3
117	gamma-Nonalactone	104-61-0	2781	172.515	178c	1.3
118	Oakmoss absolute	9000-50-4	2795	172.510	194n	1.3
119	Orris absolute	8002-73-1	N.A.	172.510	241n	1.3
120	Palmitic acid	57-10-3	2832	172.860	14c	1.3
121	Phenethyl phenylacetate	102-20-5	2866	172.515	234c	1.3
122	3-Propylidenephthalide	17369-59-4	2952	172.515	494c	1.3
123	Sage oil	8022-56-8	3001	182.20	61n	1.3
124	alpha-Terpineol	98-55-5	3045	172.515	62c	1.3
125	Terpinyl acetate	80-26-2	3047	172.515	205c	1.3
126	gamma-Undecalactone	104-67-6	3091	172.515	179c	1.3
127	gamma-Valerolactone	108-29-2	3103	N.A.	757c	1.3
128	3-Butylidenphthalide	551-08-6	3333	N.A.	N.A.	1.04
129	Davana oil	8016-03-3	2359	172.510	69n	0.65
130	3,5-Dimethyl-1, 2-cyclopentanedione	13494-07-0	3269	N.A.	2235c	0.65
131	Ethyl cinnamate	103-36-6	2430	172.515	323c	0.65
132	Farnesol	4602-84-0	2478	172.515	78c	0.65
133	Geranyl phenylacetate	102-22-7	2516	172.515	231c	0.65
134	alpha-Irone	79-69-6	2597	172.515	145c	0.65
135	Jasmine absolute	8022-96-6	2598	182.20	245n	0.65
136	Kola nut tincture	68916-19-8	2607	182.20	149n	0.65
137	Linalool oxide	1365-19-1	3746	172.515	N.A.	0.65
138	Linalyl acetate	115-95-7	2636	182.60	203c	0.65
139	*para*-Methoxybenzaldehyde	123-11-5	2670	172.515	103c	0.65

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TABLE 2
Ingredients added to test cigarettes in study 1 *(Continued)*

	Ingredient	CAS no.[a]	FEMA no.[b]	CFR[c]	CoE[d]	Application rate (ppm)
140	2-Methylbutyric acid	116-53-0	2695	172.515	2002c	0.65
141	Myristic acid	544-63-8	2764	172.860	16c	0.65
142	gamma-Octalactone	104-50-7	2796	172.515	2274c	0.65
143	Opoponax oil	8021-36-1	N.A.	172.510	313n	0.65
144	Tagetes oil	8016-84-0	3040	172.510	443n	0.65
145	3-Ethyl-2-hydroxy-2-cyclopenten-1-one	21835-01-8	3152	N.A.	759c	0.52
146	4-Methylacetophenone	122-00-9	2677	172.515	156c	0.26
147	Isobutyraldehyde	78-84-2	2220	172.515	92c	0.13
148	3-Methylbutyraldehyde	590-86-3	2692	172.515	94c	0.13
149	2,3-Dimethylpyrazine	5910-89-4	3271	N.A.	N.A.	0.13
150	2,5-Dimethylpyrazine	123-32-0	3272	N.A.	2210c	0.13
151	2,6-Dimethylpyrazine	108-50-9	3273	N.A.	2211c	0.13
152	Dimethyltetrahydrobenzofuranone	13341-72-5	3764	N.A.	N.A.	0.13
153	4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone	3658-77-3	3174	N.A.	536c	0.13
154	4-(*para*-Hydroxyphenyl)-2-butanone	5471-51-2	2588	172.515	755c	0.13
155	alpha-Ionone	127-41-3	2594	172.515	141c	0.13
156	beta-Ionone	8013-90-9	2595	172.515	142c	0.13
157	Isovaleric acid	503-74-2	3102	172.515	8c	0.13
158	Lime oil	8008-26-2	2631	182.20	141n	0.13
159	Mace absolute	8007-12-3	N.A.	182.20	296n	0.13
160	Nutmeg oil	8008-45-5	2793	182.20	296n	0.13
161	Caprylic acid	124-07-2	2799	184.1025	10c	0.13
162	Phenylacetaldehyde	122-78-1	2874	172.515	116c	0.13
163	5,6,7,8-Tetrahydroquinoxaline	34413-35-9	N.A.	N.A.	721c	0.13
164	Thyme oil	8007-46-3	3064	182.20	456n	0.13
165	Valeraldehyde	110-62-3	3098	172.515	93c	0.13

Note. "n" Follows the name of natural source of flavorings and "c" follows the number of chemical substances.
[a]Chemical Abstract Service registry number.
[b]The Flavor and Extract Manufacturers Association reference number.
[c]Code of Federal Regulations reference to Title 21 indicating regulatory status of material.
[d]Council of Europe reference number.

Inhalation Toxicity Study Design

Groups of 30 Sprague-Dawley rats of each sex were exposed by nose-only inhalation for 1 h/day, 5 days/wk for 13 consecutive weeks to concentrations of 0.06, 0.2, or 0.8 mg/L WTPM of smoke from test cigarettes containing flavoring (study 1) or to flavoring or casing ingredients (study 2). Additional groups of 30 rats/sex were exposed to the same concentrations of smoke from reference cigarettes, similar to the test cigarettes but without the flavoring or casing ingredients (as described above), or to filtered air only (sham controls). This exposure regimen (1 h/day, 5 days/wk) reflects current laboratory practices for animal inhalation studies comparing the effects of smoke from test and reference cigarettes, and does not simulate human usage patterns. However, this difference should not influence the validity of the results.

Each group of 30 rats/sex was subdivided into 2 groups: 20 rats/sex scheduled for necropsy immediately after 13 wk of exposure (interim sacrifice) and up to 10 rats/sex scheduled for necropsy following 13 wk of recovery from smoke exposure (final sacrifice). Target smoke concentrations were 0.06, 0.2, or 0.8 mg WTPM/L for the test and reference cigarettes. An additional group of 30 rats/sex served as sham controls.

Biological endpoints for the 13-wk exposure and 13-wk recovery groups included clinical appearance, body weight, organ weights, and gross and microscopic lesions. Plasma nicotine, COHb, and respiratory parameters were measured periodically during the 13-wk exposure period and clinical pathology parameters were measured at the end of the 13-wk exposure period.

Smoke Generation and Exposure System

Animal exposures were conducted in AMESA exposure units (C. H. Technologies, Westwood, NJ). The smoke exposure machines were designed to contain 30 cigarettes on a smoking head that rotated 1 revolution per minute (Baumgartner and Coggins,

TABLE 3
Ingredients added to study 2 test cigarettes

	Ingredient	CAS no.[a]	FEMA no.[b]	CFR[c]	CoE[d]	Application rate (ppm)
1	Invert sugar	8013-17-0	N.A.	184-1859	N.A.	20,000
2	Block chocolate	N.A.	N.A.	N.A.	N.A.	2,500
3	Plum extract	90082-87-4	N.A.	N.A.	371n	2,200
4	Fig extract	90028-74-3	N.A.	N.A.	198n	2,000
5	Molasse extract and tincture	68476-78-8	N.A.	N.A.	371n	2,000
6	Gentian root extract	97676-22-7	2506	172-510	214n	1,000
7	Lovage extract	8016-31-7	2650	172-510	261n	1,000
8	Peppermint oil	8006-90-4	2848	182-20	282n	250

Note. "n" Follows the name of natural source of flavorings and "c" follows the number of chemical substances.
[a]Chemical Abstract Service registry number.
[b]The Flavor and Extract Manufacturer's Association reference number.
[c]Code of Federal Regulations reference to Title 21 indicating regulatory status of material.
[d]Council of Europe reference number.

1980; Ayres et al., 1990). A vacuum port aligned with, and drew a puff from, one test or reference cigarette at a time as the head rotated. Air was drawn through the vacuum port by a peristaltic pump operating at a flow rate of ~1.05 L/min, creating a 2-s, 35-ml puff through each cigarette once each minute. The smoke vacuum flow rate was regulated by a concentration control unit consisting of a real-time aerosol monitor [(RAM)-1; MIE, Inc., Bedford, MA], a computer, and an electronic flow controller (Emerson Electric Co., Brooks Instrument Division, Hatfield, PA). The computer monitored analog voltage output of the RAM and adjusted the amount of smoke that was drawn from the glass mixing bowl by the flow controller until RAM voltage matched the calculated target voltage. The exposure units contained 3 tiers, each with 24 animal exposure ports. The exposure ports were connected to a delivery manifold, which transferred smoke to the animal breathing zone, and to an outer concentric manifold that drew the exhaled and excess smoke to an exhaust duct. Each cigarette was retained for seven puffs.

Exposure Atmosphere Characterization

The protocol-prescribed limits for the smoke concentration (WTPM/L) were target ±10% coefficient of variation (%CV). Smoke exposure concentrations were continuously monitored with a RAM at a representative exposure port. Mean exposure concentration was calculated from the mass collected on the filter and the total volume of air drawn through the filter, which was determined by the sample time and flow rate. RAM voltage readings were recorded during filter sample collection and were used to calculate a RAM response factor for subsequent exposures.

Two filters per exposure group per week were chemically analyzed for total nicotine. Nicotine standard reference material (98%) was purchased from Aldrich Chemical Company, Inc. (Milwaukee, WI). The WTPM:nicotine and CO:nicotine ratios were calculated for the exposure atmospheres. The concentration of CO in the test and reference atmospheres was determined using Horiba PIR-2000 CO analyzers (Horiba Instruments, Inc., Irvine, CA), monitored by DOS-based computers.

Particle size distribution of the smoke was measured using Mercer-style cascade impactors designed specifically for the size range of particles found in cigarette smoke. The mass collected on each impactor stage was analyzed gravimetrically for WTPM and the resulting data were interpreted by probit analysis (NEWCAS; Hill et al., 1977) to obtain the particle size distribution, mass median aerodynamic diameter (MMAD), and geometric standard deviation (GSD). Temperature and RH of the exposure atmospheres were measured from a representative animal exposure port once every 2 wk for each exposure group.

Animals and Animal Care

Sprague-Dawley (Crl:CD) rats 4–5 wk of age were purchased from Charles River Laboratories (Raleigh, NC), held for 13 days in quarantine status prior to initial smoke exposure. Health screens were performed following group assignment and at 24 days after arrival. These health evaluations included necropsy, microscopic examination of selected tissues and examination for parasites. The 24 days after arrival screening included serological testing for antibodies to common viral pathogens. Viral antibody testing was also performed on sera collected from 10 sentinel rats at the end of the 13-wk exposure period and from another 10 at the end of the recovery period. All sera were tested for antibodies to Sendai virus, Kilham's rat virus (KRV)/Toolan's H-1 virus, pneumonia virus of mice (PVM), rat corona virus/sialodacryoadenitis virus, and *Mycoplasma pulmonis*. During the 13-wk exposure period, the animals were housed in individual stainless-steel cages on open racks. During the recovery period, the animals were housed in individual polycarbonate cages (Lab Products, Maywood, NJ) bedded with

ALPHA-dri alpha cellulose bedding (Sheperd Specialty Papers, Kalamazoo, MI). The cage space met the requirements stated in the current *Guide for Care and Use of Laboratory Animals* (National Academy of Sciences, 1996).

Body Weight and Clinical Observations

All rats were observed twice daily for mortality and moribundity. Each rat was examined every 4 wk for clinical signs. Individual body weights were measured during the randomization procedure, on exposure day 1, biweekly thereafter, and at necropsy.

Respiratory Function Measurements

Tidal volume (TV), respiratory rate (RR), and minute volume (MV), derived from flow signals from spontaneously breathing animals, were measured in 4 rats/sex/group during wk 2, 8, and 13 using whole-body phethysmography (Coggins et al., 1981). Each animal was monitored once during a single exposure period. MV and the actual WTPM were used to estimate the average total inhaled mass for the 1-h exposure period for each animal.

Carboxyhemoglobin and Plasma Nicotine Determinations

During wk 2 and 10, blood was collected from designated animals at the end of the 1-h smoke exposure. Animals were removed from the exposure unit and bleeding was initiated within ~5 min. The blood samples were obtained from the retro-orbital plexus of carbon dioxide (CO_2)-anesthetized animals into tubes containing potassium ethylenediaminete traacetic acid (K^+-EDTA). The sample tubes were immediately placed into an ice bath and maintained under these conditions until analyzed for blood carboxyhemoglobin (COHb). Plasma nicotine was quantitatively determined using gas chromatography/mass spectrometry (GC/MS) with selected ion monitoring.

Clinical Pathology

On the day of the 13-wk interim sacrifice, the rats were anesthetized with ~70% CO_2 in room air and blood samples were obtained from the retro-orbital plexus. One sample was collected in a tube (Monoject, Sherwood Medical, St. Louis, MO) containing K^+-EDTA for hematologic determinations. Another sample was collected in a tube devoid of anticoagulant but containing a separator gel (Vacutainer, Franklin Lakes, NJ) for serum chemistry analysis. The following parameters were determined using an Abbott Cell-Dyn 3700 (Abbott Diagnostics Systems, Abbott Park, IL) multiparameter hematology instrument: white blood cell (WBC) count, red blood cell (RBC) count, hemoglobin (Hb) concentration, volume of packed red cells (VPRC), the red cell indices (mean corpuscular volume [MCV], mean corpuscular hemoglobin [MCH], and mean corpuscular hemoglobin concentration [MCHC]), platelet count, and WBC differential counts. Results of the differential cell counts were reported as both relative and absolute values. Reticulocytes were stained supravitally with new methylene blue and enumerated as reticulocytes per 1000 enthrocytes using the Miller disc method (Brecher and Schneiderman, 1950).

A Roche Hitachi 912 system (Roche Diagnostic Corp., Indianapolis, IN) chemistry analyzer was used to determine the following serum analytes: urea nitrogen (BUN), creatinine, glucose, total protein, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transpeptidase (GGT), sodium, potassium, chloride, calcium, phosphorus, total bilirubin, cholesterol, and triglycerides.

Necropsy and Tissue Collection

A complete necropsy was done on all 13-wk exposure groups and 13-wk recovery group animals. Rats designated for scheduled sacrifices or sacrificed due to moribund condition were weighed and anesthetized with 70% CO_2 in air, followed by exsanguination before cessation of heartbeat. All abnormalities were recorded on the individual animal necropsy forms. Lungs, liver, kidneys, testes, adrenals, spleen, brain, and heart from all scheduled sacrifice animals were weighed. These organ weights and the body weights at necropsy were used to calculate organ:body weight ratios. In addition, organ:brain weight ratios were calculated. The time from removal of the organ until weighing was minimized to keep tissues moist.

A complete set of over 40 tissues was removed from each animal at necropsy and examined. All tissues were fixed in 10% neutral buffered formalin (NBF) except for the eyes, which were fixed in Karnovsky's fixative. After the lungs were weighed, they were perfused with 10% NBF at 25 cm hydrostatic pressure.

Histopathology

All tissues were fixed in 10% NBF for a minimum of 48 h before being trimmed. Paraffin blocks were microtomed at 5 μm. All sections were stained with hematoxylin and eosin (H&E) stains for standard histopathologic evaluation of morphologic changes. Duplicate slides of nasal tissues, larynx, lung, and trachea were stained with periodic acid-Schiff/Alcian blue (PAS/AB) stains for evaluation of goblet cell populations. The lungs, nasal cavity (four sections), nasopharynx, larynx (three cross sections), trachea (three transverse sections), tracheobronchial lymph nodes, mediastinal (thymic) lymph nodes, heart, and all gross lesions were examined microscopically. The lungs were sectioned to present a maximal section of the mainstem bronchi. The nasal cavity was prepared in four sections using the landmarks described by Young (1981). Three transverse laryngeal sections were prepared from the base of the epiglottis, the ventral pouch, and through the caudal larynx at the level of the vocal folds (Renne et al., 1992). In addition, sections of brain, adrenals, spleen, liver, kidneys, and gonads from animals in the sham control and the groups exposed to 0.8 mg/L of smoke from the test or reference cigarettes were examined microscopically. Exposure-related microscopic lesions were observed in the tissues from the rats exposed to 0.8 mg/L; target organs were examined microscopically in the lower concentration groups to ascertain a no-effect concentration.

Evaluation of Cell Proliferation Rates of Respiratory-Tract Tissues

Cell proliferation rates were measured on respiratory tract tissues collected from 10 rats of each sex from each exposure group and the sham controls necropsied immediately after 13 wk of exposure, using a monoclonal antibody to 5-bromo-2′-deoxyuridine (BrdU). Tissues evaluated using the BrdU assay included the respiratory epithelium lining the median nasal septum and distal portions of maxillary and nasal turbinates, the transitional epithelium at the base of the epiglottis, the luminal epithelium dorsolateral to the ventral pouch, the luminal epithelium lining the cranial trachea, the luminal epithelium of the mainstem bronchi and adjacent bronchioles, and selected areas of alveolar epithelium. Data from both sides of bilaterally symmetrical tissues (nose, ventral pouch, mainstem bronchi) were combined for tabulation of results.

Statistical Methods

Body weight, body weight gain, organ:body weight, and organ:brain weight ratios were statistically analyzed for each sex by exposure concentration group using the Xybion PATH/TOX system. Data homogeneity was determined by Bartlett's test. Dunnett's t-test was performed on homogeneous data to identify differences between each concentration group and the sham control group, and between corresponding concentrations of test and reference cigarette smoke-exposed groups. Nonhomogeneous data were analyzed using a modified t-test. Respiratory physiology, clinical pathology, COHb, and plasma nicotine data parameters were statistically evaluated using SAS software (Statistical Analysis System, SAS, Inc., Cary, NC). One-way analysis of variance (ANOVA) between exposure groups was first conducted, followed by Bartlett's test for homogeneity of variance. A two-sided Dunnett's multiple comparison test was employed to determine which exposure groups were different from the controls. An unpaired two-sided t-test was used to compare equivalent exposure groups between cigarette types. Differences were considered significant at $p \leq .05$. The statistical evaluation of incidence and severity of lesions was made using the Kolmogorov–Smirnov two-sample test (Siegel, 1956). All treatment group means were compared to the sham control mean, and means of groups exposed to the test cigarette smoke were compared to the corresponding reference cigarette smoke-exposed group means. Cell proliferation data were compared statistically using Tukey's studentized range test with SAS software.

RESULTS

Cigarette Performance

The results of characterization of the test and reference cigarettes for study 1 and study 2 are presented in Tables 4 and 5. These results show that the filler weight and the number of puffs per cigarette, nicotine yield, and nicotine-free dry particulate matter (NFDPM) were comparable for test and reference cigarettes in both studies. The yields of nicotine and NFDPM and the puff count were also comparable. These results are consistent with the negligible differences in the configuration of both prototype cigarettes, which basically consist of the total relative amount of flavor ingredient contained in the test cigarettes (1% or 3% of the filler weight). A comparison of the burning rates in study 1 illustrates that the addition of the ingredients had little, if any effect on the burning characteristics of the test cigarettes.

TABLE 4
Key parameters for laboratory control of prototype study 1 cigarettes

Parameter	Target	Run average: Test cigarette	Run average: Reference cigarette
Individual weights (g)			
Cigarette weight	1.012	0.963	0.965
Standard deviation	—	0.019	0.018
Non tobacco weight	0.212	0.212	0.215
Net tobacco	0.800	0.751	0.750
Air dilution (%)	32	35	34.1
Standard deviation	—	3.0	3.1
Porosity of cigarette paper (cc/min/cbar/cm^2)	50	49	49
Expanded tobacco (%)	9.7	10.1	9.1
Nicotine (mg/cig)	0.9	0.92	0.97
Nicotine (mg/puff)	n.a.	0.118	0.123
NFDPM (mg/cig)	12.0	11.3	11.5
NFDPM (mg/puff)	n.a.	1.45	1.46
CO (mg/cig)	n.a.	12.4	13.1
CO (mg/puff)	n.a.	1.59	1.66
Puffs/cigarette	n.a.	7.8	7.9
Burning rate (mg tobacco/min)	n.a.	68.1	64.4

Note. Cig, cigarette.

In Vitro Mutagenicity Assays

Figures 1, 2, 3, and 4 summarize the results of Ames assays on test cigarettes from study 1 and 2 with and without metabolic activation. TA100, TA98, and TA1537 strains showed a positive response only with metabolic activation. No response was observed in TA 102 or TA1535. No sporadic responses in revertants were recorded. The highest sensitivity and specificity of the mutagenic response were observed using TA98 with metabolic activation. From the comparison of the data obtained for the test and reference cigarettes, it was concluded that the addition of ingredients did not result in a positive mutagenic response in any of the strains under the conditions already described. Hence, the use of the tested ingredients had no influence on the mutagenic activity of the cigarettes.

TABLE 5
Key parameters for laboratory control of prototype study 2 cigarettes

Parameter	Target	Run average	
		Test cigarette	Reference cigarette
Individual weights (g)			
Cigarette weight	1.012	1.002	1.025
Standard deviation	—	0.0208	0.0173
Nontobacco weight	0.212	0.212	0.212
Net tobacco	0.800	0.790	0.813
Air dilution (%)	32	33.2	36.6
Standard deviation	—	1.6	1.4
Porosity of cigarette paper (cc/min/cbar/cm^2)	50	50	47
Expanded tobacco (%)	9.5	9.6	9.3
Nicotine (mg/cig)	0.9	0.93	0.93
Nicotine (mg/puff)	n.a.	0.112	0.107
NFDPM (mg/cig)	12.0	11.4	11.0
NFDPM (mg/puff)	n.a.	1.37	1.26
CO (mg/cig)	n.a.	12.9	12.8
CO (mg/puff)	n.a.	1.55	1.47
Puffs/cigarette	n.a.	8.3	8.7

Note. Cig, cigarette.

Exposure Atmosphere Characterization

Tables 6 and 7 summarize the exposure data for the inhalation exposure periods for study 1 and study 2. The mean exposure concentrations (WTPM) were all within 3% of the target concentration, with CVs of 6.6%, or less. Nicotine and CO concentrations correlated well with WTPM in reference and test cigarette smoke atmospheres in both study 1 and study 2. Particle sizes were slightly larger in the study 1 test and reference cigarette smokes. All concentrations of the smoke from each cigarette were highly respirable for the rat model under investigation.

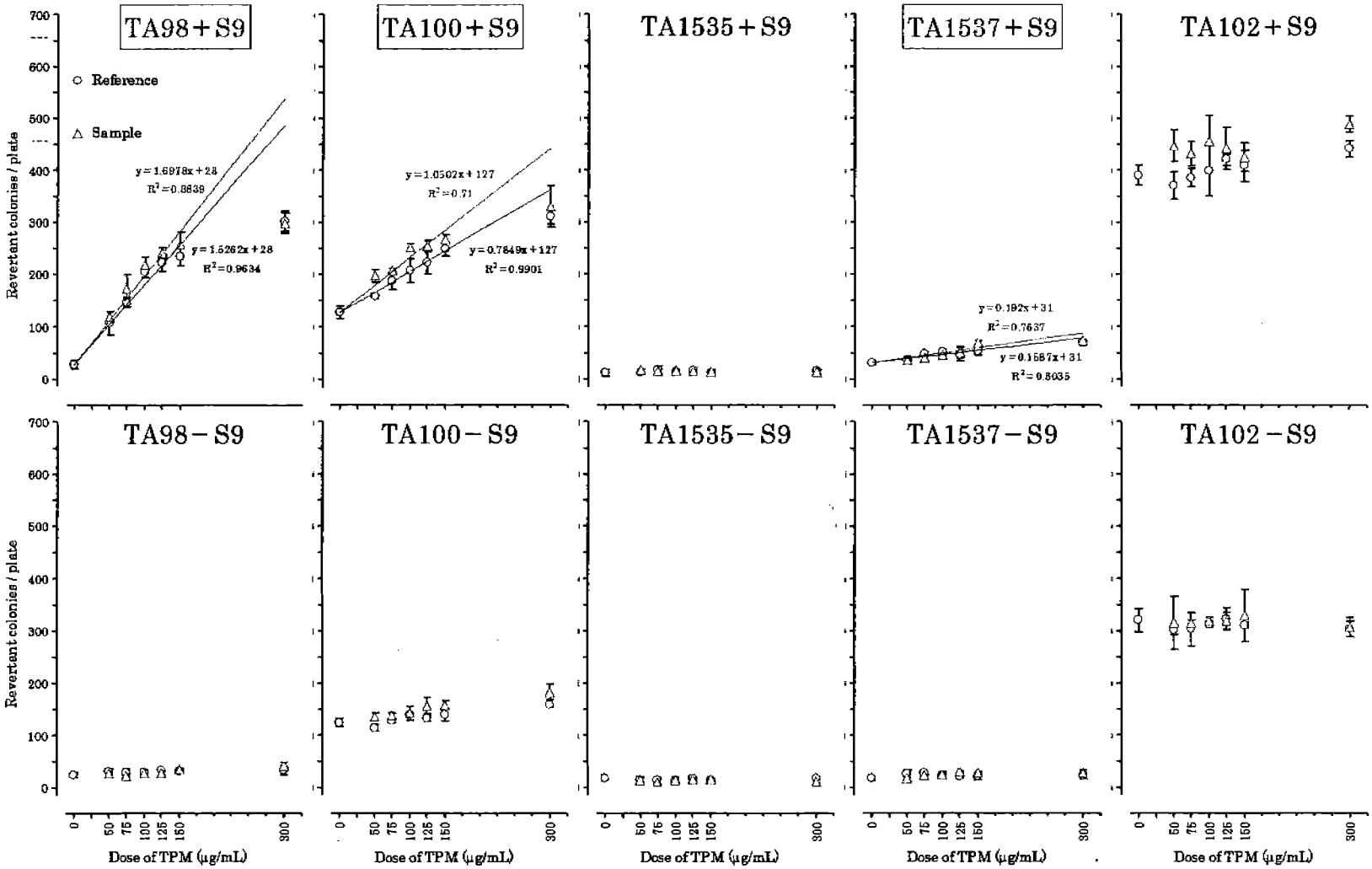
Body Weights and Clinical Observations

No significant mortality occurred in either study. Exposure-related adverse clinical signs were absent. Clinical observations noted were minor in consequence and low in incidence.

Mean body weight data for all groups on study throughout the exposure and recovery periods are illustrated in Figure 5. In study 1, mean body weights were consistently decreased compared to sham controls during the exposure period in male rats exposed to 0.8 mg/L of reference cigarette smoke and in males exposed to all 3 concentrations of test cigarette smoke. With the exception of day 71 (0.8 mg/L test), all female smoke-exposed groups in study 1 were comparable to sham control females throughout the study. In study 2, mean body weights were consistently decreased compared to sham controls in males exposed to 0.8 mg/L of test cigarette smoke and in females exposed to 0.8 mg/L of reference cigarette smoke. Mean body weights of smoke-exposed groups were similar to sham control weights during the recovery period of both study 1 and study 2. The only consistent statistical difference in body weight changes between the test and reference cigarette smoke-exposed groups in either study was the decreased mean body weight in males exposed to 0.8 mg/L of reference cigarette smoke during the exposure period of study 1.

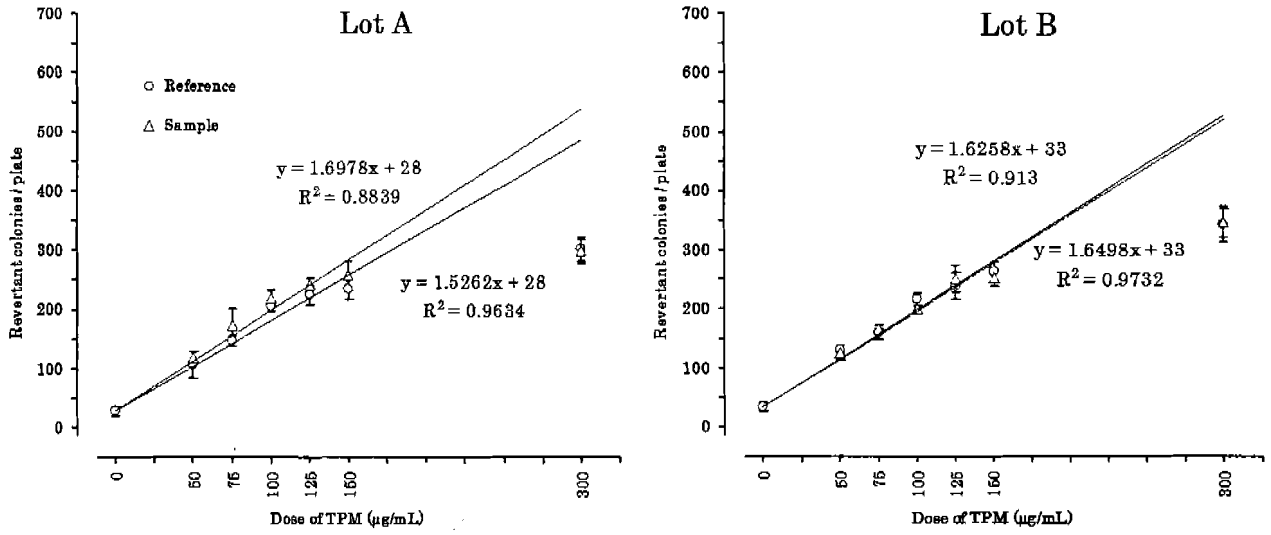
Organ Weights

Comparisons of selected group mean organ weights between smoke-exposed and sham controls in study 1 are presented in Table 8. Statistically significant differences in organ weights in groups of smoke-exposed rats were primarily low mean organ weights compared to their respective sham controls. There was no clear pattern of differences in any absolute or relative organ weight in smoke-exposed groups compared to sham controls, or in groups exposed to test versus reference cigarette smoke at either the interim sacrifice or the recovery sacrifices. Sham controls for the interim sacrifice of study 2 were inadvertently not fasted overnight prior to necropsy, which made comparison of absolute and relative organ weights of smoke-exposed and sham control groups from the interim sacrifice of questionable scientific value; thus these comparisons were not made for study 2. Statistical comparison of absolute and relative organ weights between groups exposed to test and reference cigarette smoke in study 2 showed very few statistically significant differences, none of which were considered toxicologically



N=2. Only the first lot (Lot A) is indicated in this figure.
The second lot (Lot B) showed the same tendency as the first lot.

FIG. 1. Ames assay results, study 1 cigarettes.



MEAN ± SD of Specific Activity (50 to 150 µg/plate)

Reference	1576 ± 141.9	Reference.....	1734 ± 170.9
Sample.....	1783 ± 167.3	Sample.....	1703 ± 151.2

FIG. 2. Ames assay results, study 1 with TA98 metabolic activation.

significant. Comparison of organ weights in rats necropsied following the 13-wk recovery of study 2 indicated no consistent differences between sham control and smoke-exposed groups, or between groups exposed to similar concentrations of test and reference cigarette smoke.

Respiratory Physiology

Reductions in RR and/or TV resulted in consistently lower MV in rats exposed to test or reference cigarette smoke compared to sham controls in both study 1 and study 2. There was no consistent difference in MV between groups of rats exposed to test and reference cigarette smoke in either study. Because the overall MV in study 1 was similar among groups exposed to smoke, total inhaled mass was proportional to increasing smoke concentration in this study. In study 2, decreases in MV in groups exposed to 0.8 or 0.2 mg/L compared to groups exposed to 0.06 mg/L caused total inhaled mass for the high and middle dose groups to be lower in proportion to the exposure concentration of inhaled smoke.

Clinical Pathology

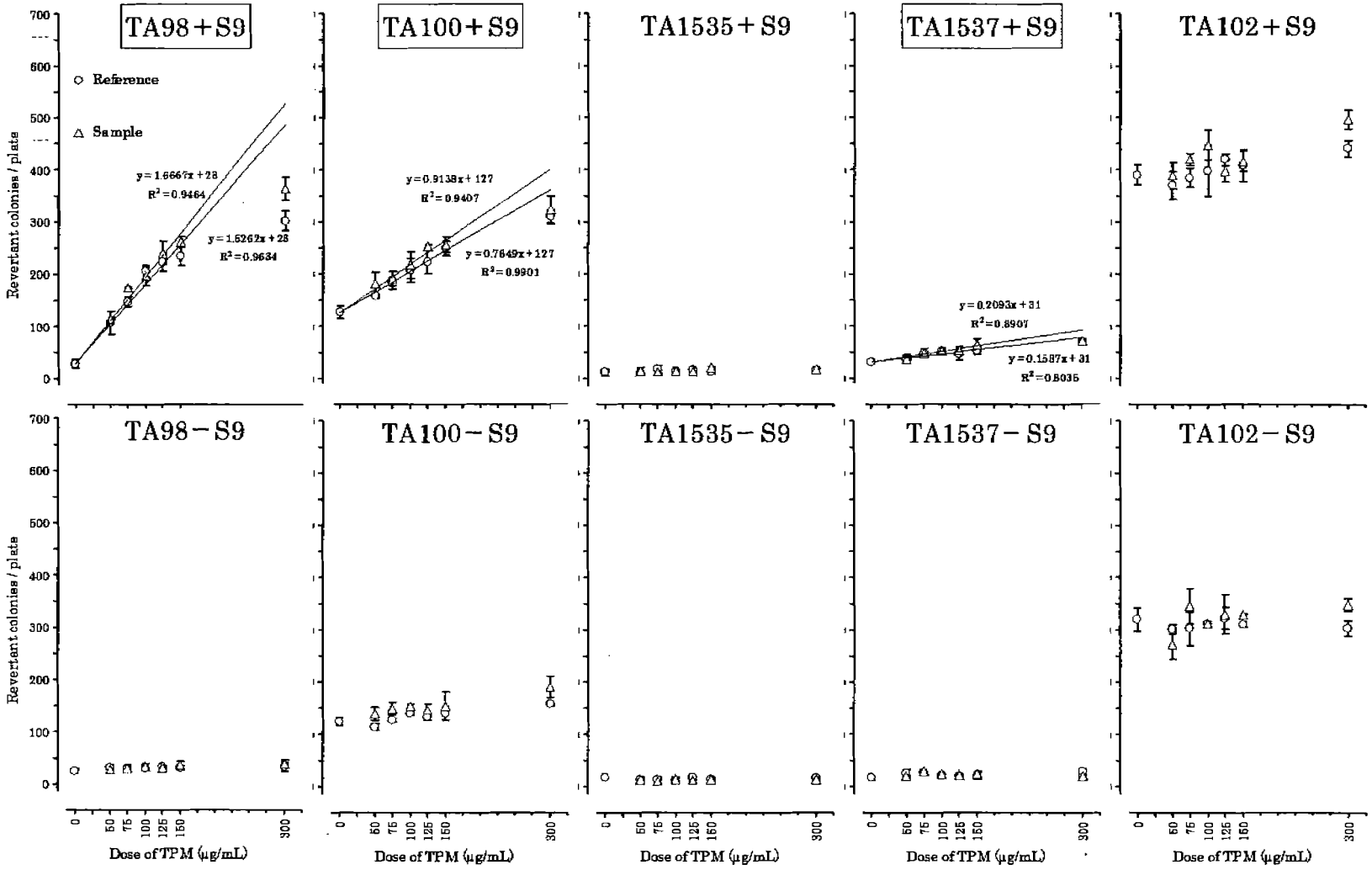
There were occasional statistically significant differences in hematology and clinical chemistry parameters from control values in groups exposed to smoke from test or reference cigarettes in both study 1 and study 2. These differences did not occur in a dose-response pattern and were well within ±2 standard deviations of historic values for control Sprague-Dawley rats of comparable age. There were also statistically significant differences in several hematology and clinical chemistry parameters between groups exposed to similar concentrations of test and reference cigarette smoke. These differences are not considered to be of toxicologic significance, nor were they exposure related.

Whole-blood COHb levels were increased in a graded dose-response fashion as a function of exposure concentration for all test and reference cigarette smoke-exposed groups in both studies. In study 2 rats bled during exposure wk 2, there was a statistically significant decrease in COHb levels in both sexes exposed to 0.8 mg/L of test cigarette smoke and in females exposed to 0.2 mg/L of test cigarette smoke, compared to groups exposed to reference cigarette smoke. There were no other clear differences in whole blood COHb levels between the test and reference cigarette groups at equivalent exposure levels in either study.

Plasma nicotine levels increased in a graded dose-response fashion for test and reference males and female groups in both studies. In study 2, test female groups exposed to 0.8 mg/L had significantly lower plasma nicotine levels than the 0.8 mg/L reference females at both 2- and 10-wk sampling. Comparing males to females at all exposure levels for test and reference cigarettes, the females consistently had higher plasma nicotine levels in both studies.

Pathology

Few gross lesions were observed in either study, with no evidence of changes attributable to exposure to smoke from the test



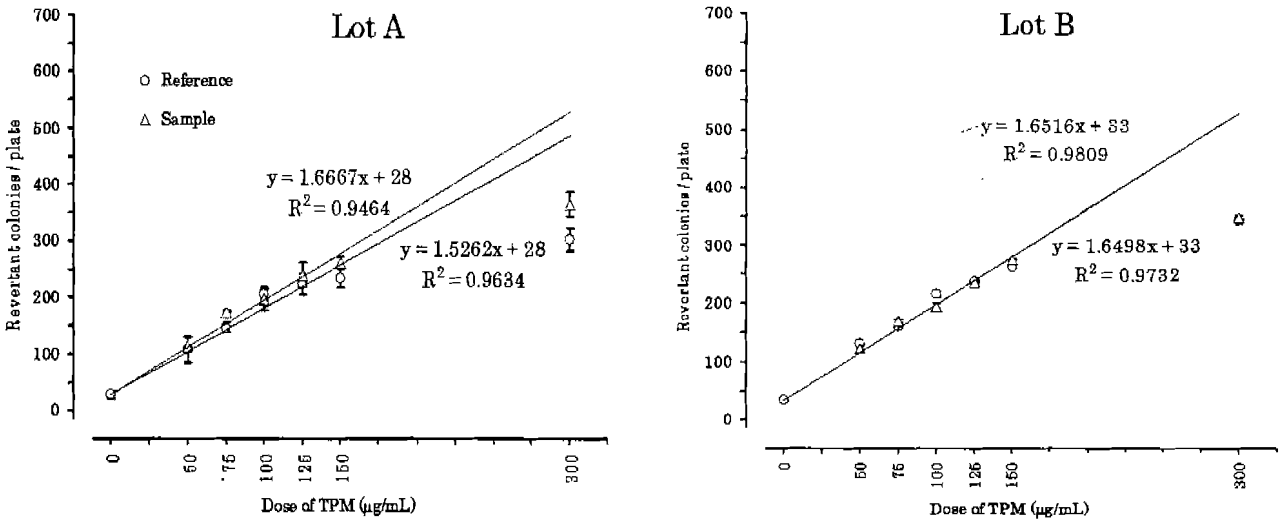
N=2. Only the first lot (Lot A) is indicated in this figure.
The second lot (Lot B) showed the same tendency as the first lot .

FIG. 3. Ames assay results, study 2 cigarettes.

TABLE 6
Study 1, exposure concentration data for rats exposed to mainstream smoke from test or reference cigarettes

	Concentration [mean ± SD (%CV)]				
	Measured exposure concentration (mg WTPM/L; $n = 126$)	Nicotine concentration (μg/L; $n = 28$)	CO concentration (ppm; $n = 63$)	Percent of target WTPM concentration (mean ± SD)	Particle size (MMAD, μm)
Test target exposure concentration (mg WTPM/L)					
0.800	0.787 ± 0.035 (4.4)	68.2 ± 2.5 (3.7)	584 ± 27 (4.6)	98.4 ± 4.3	0.73 ± 0.08
0.200	0.199 ± 0.009 (4.5)	15.5 ± 1.0 (6.5)	144 ± 6 (4.2)	99.3 ± 4.3	0.74 ± 0.12
0.060	0.061 ± 0.004 (6.6)	4.4 ± 0.5 (11.4)	47 ± 3 (6.4)	101 ± 6	0.69 ± 0.09
Reference target exposure concentration (mg WTPM/L)					
0.800	0.795 ± 0.023 (2.9)	70.1 ± 2.1 (2.9)	608 ± 20 (3.3)	99.4 ± 2.7	0.74 ± 0.08
0.200	0.202 ± 0.004 (2.0)	15.8 ± 0.7 (4.5)	147 ± 4 (2.7)	101 ± 2	0.72 ± 0.07
0.060	0.060 ± 0.002 (3.3)	4.4 ± 0.4 (9.8)	50 ± 2 (4.8)	100 ± 4	0.74 ± 0.10

Note. CO, carbon monoxide; WTPM, wet total particulate matter.



MEAN±SD of Specific Activity (50 to 150 µg/plate)

Reference.....	1576±141.9	Reference.....	1734±170.9
Sample.....	1726±138.6	Sample-1.....	1701±107.9

FIG. 4. Ames assay results, study 2 cigarettes with TA98 metabolic activation.

TABLE 7
Study 2, exposure concentration data for rats exposed to smoke from test or reference cigarettes

	Concentration [mean ± SD (%CV)]				
	Measured exposure concentration (mg WTPM/L; $n = 134$)	Nicotine concentration (μg/L; $n = 28$)	CO concentration (ppm; $n = 67$)	Percent of target WTPM concentration (mean ± SD)	Particle size (MMAD, μm)
Test target exposure concentration (mg WTPM/L)					
0.8	0.798 ± 0.040 (5.0)	56.8 ± 2.6 (4.6)	646 ± 34 (5.3)	100 ± 5	0.65 ± 0.01
0.2	0.194 ± 0.007 (3.6)	12.9 ± 0.6 (4.7)	158 ± 9 (5.7)	97 ± 4	0.62 ± 0.04
0.060	0.060 ± 0.002 (3.3)	4.0 ± 0.2 (5.0)	54 ± 3 (5.6)	100 ± 3	0.66 ± 0.03
Reference target exposure concentration (mg WTPM/L)					
0.8	0.784 ± 0.031 (4.0)	55.1 ± 2.3 (4.2)	676 ± 31 (4.6)	98 ± 4	0.57 ± 0.03
0.2	0.201 ± 0.004 (1.8)	13.0 ± 0.4 (3.4)	170 ± 15 (8.7)	100 ± 2	0.64 ± 0.07
0.060	0.060 ± 0.002 (3.3)	4.1 ± 0.2 (4.4)	57 ± 3 (5.8)	99 ± 3	0.66 ± 0.06

Note. CO, carbon monoxide; WTPM, wet total particulate matter.

or the reference cigarettes. Exposure to smoke from reference or test cigarettes in both studies induced concentration-related proliferative, metaplastic, and inflammatory microscopic lesions in the respiratory tract after 13 wk of exposure. The incidence of exposure-related respiratory-tract lesions observed at microscopic examination of tissues from rats necropsied at the interim sacrifice immediately following 13 wk of exposure is summarized in Table 9 for study 1 and Table 10 for study 2.

Hyperplasia of respiratory epithelium lining the anterior nasal cavity was present in all rats exposed to 0.8 mg/L in both studies, a few rats exposed to 0.2 mg/L in both studies, and in 3/40 rats exposed to 0.06 mg/L in study 1. Areas most severely and most frequently affected were the distal portions of the nasal and maxillary turbinates in sections of nose just caudal to the incisor teeth. In affected rats, the epithelium in the distal turbinates was up to six cells thick. There was also a clear dose response in the severity of nasal respiratory epithelial hyperplasia, with severity ranging from minimal to moderate. Comparison of incidence and severity data for nasal respiratory epithelial hyperplasia in rats exposed to similar concentrations of smoke from the test and reference cigarettes did not indicate any statistically significant differences in either study. Minimal goblet-cell hyperplasia was observed in the mucosal epithelium lining the median nasal septum in some smoke-exposed and sham control rats. Although not statistically significant compared to concurrent sham controls, the incidence of nasal goblet cell hyperplasia in male rats exposed to the 0.8-mg/L concentration of smoke from the reference cigarette or test cigarette in study 1 were considered to be toxicologically significant. There was no clear difference in the incidence of goblet cell hyperplasia between groups exposed to similar concentrations of reference and test cigarette smoke in either study.

Exposure to smoke from the reference or test cigarette in both study 1 and study 2 induced squamous metaplasia, hyperplasia, and hyperkeratosis of the transitional epithelium lining the base of the epiglottis and the epithelium lining the dorsal border of the ventral pouch and the adjacent laryngeal lumen. In control rats, the epithelium lining the base of the epiglottis was a mixture of ciliated columnar epithelium and slightly flattened, oval, rounded, or cuboidal cells one or two cells thick over a poorly defined basal cell layer (Renne et al., 1992). In affected smoke-exposed rats, the base of the epiglottis was covered by a stratified squamous epithelium up to eight cells thick with a variably keratinized surface layer and a distinct basal cell layer. There was a concentration-related increase in severity of squamous metaplasia and hyperplasia of epiglottis epithelium in rats exposed to test or reference cigarette smoke. Statistical analysis did not indicate any significant differences in incidence or severity of these lesions between test and reference cigarette smoke-exposed groups in either study. Hyperkeratosis (accumulation of keratinized squamous cells on the surface) was observed in association with squamous metaplasia of the epithelium lining the base of the epiglottis in most rats exposed to smoke from reference or test cigarettes. Comparison of incidence/severity of hyperkeratosis in the epiglottis between test and reference cigarette smoke-exposed groups indicated a statistically

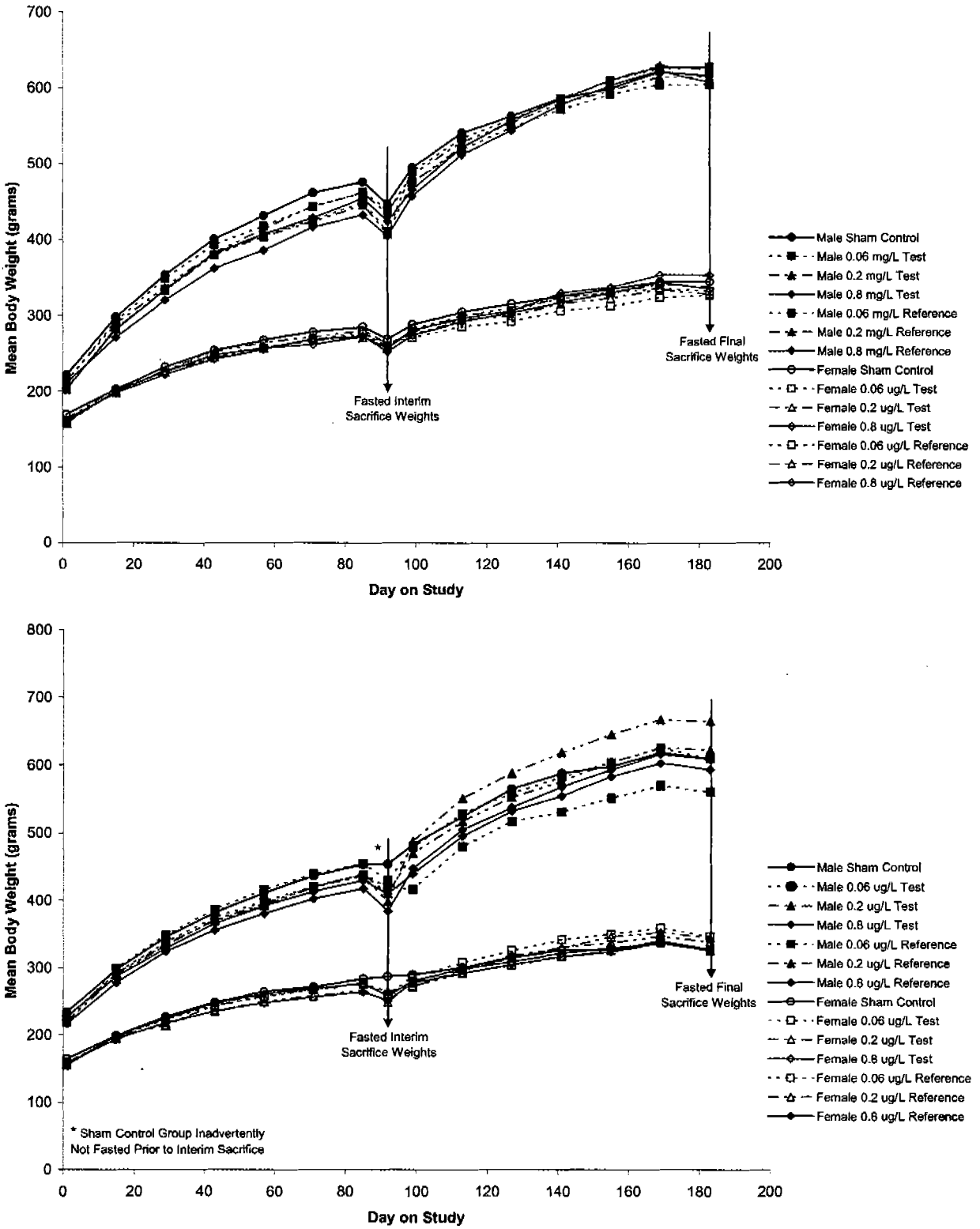


FIG. 5. Body weights, study 1 (top) and study 2 (bottom).

TABLE 8
Organ weights for rats exposed to smoke from study 1 cigarettes ($n = 20$, g ± SD)

		Test			Reference		
	Sham control	0.06 mg WTPM/L	0.2 mg WTPM/L	0.8 mg WTPM/L	0.06 mg WTPM/L	0.2 mg WTPM/L	0.8 mg WTPM/L
Males							
Heart	1.60 ± 0.16	1.48 ± 0.15[a,b]	1.43 ± 0.16[a,c]	1.55 ± 0.15	1.60 ± 0.13	1.57 ± 0.16	1.52 ± 0.15
Kidneys	3.39 ± 0.33	3.17 ± 0.39	2.92 ± 0.30[a,c]	3.05 ± 0.33[a]	3.38 ± 0.33	3.20 ± 0.31	3.02 ± 0.27[a]
Lungs	1.95 ± 0.22	1.89 ± 0.17	1.82 ± 0.23[c]	1.93 ± 0.14	2.02 ± 0.28	1.98 ± 0.26	1.89 ± 0.15
Adrenals	0.066 ± 0.010	0.066 ± 0.012	0.059 ± 0.010	0.064 ± 0.012	0.062 ± 0.007	0.064 ± 0.008	0.063 ± 0.008
Females							
Heart	1.06 ± 0.09	1.02 ± 0.10	1.00 ± 0.10[c]	1.05 ± 0.12	1.03 ± 0.09	1.07 ± 0.09	1.09 ± 0.12
Kidneys	2.18 ± 0.21	2.02 ± 0.24	1.90 ± 0.19[a]	1.93 ± 0.18[a]	2.04 ± 0.21	1.99 ± 0.19[a]	1.95 ± 0.19[a]
Lungs	153 ± 0.13	1.50 ± 0.13	1.52 ± 0.17[c]	1.52 ± 0.15	1.55 ± 0.14	1.50 ± 0.17	1.60 ± 0.19
Adrenals	0.080 ± 0.010	0.081 ± 0.011	0.078 ± 0.008	0.082 ± 0.012	0.078 ± 0.008	0.080 ± 0.010	0.081 ± 0.013

[a] $p < .05$, Dunnett's t-test of significance, compared to sham control.
[b] $p < .05$, Dunnett's t-test of significance, compared to 0.06 reference group.
[c] $p < .05$, Dunnett's t-test of significance, compared to 0.2 reference group.

significant difference only in the 0.06-mg/L groups from study 1, in which females exposed to test cigarette smoke had a higher incidence/severity than females exposed to reference cigarette smoke. Chronic inflammation was present in the submucosa of the epiglottis in some rats exposed to reference or test cigarette smoke in study 1, most frequently in rats exposed to the 0.8 mg/L smoke concentration. Squamous metaplasia, hyperplasia, and hyperkeratosis were also present in the epithelium lining the opening of the ventral pouch and the adjacent laryngeal lumen in most rats exposed to smoke from the test or reference cigarette in both studies. In control rats, the epithelium lining the opening of the ventral pouch and adjacent laryngeal lumen was slightly flattened, oval, rounded, or cuboidal cells one or two cells thick with no discernible basal cell layer (Renne et al., 1992). In affected smoke-exposed rats, this area was covered by a stratified squamous epithelium from three to six cells thick with a variably keratinized surface layer and a distinct basal cell layer. Comparison of incidence/severity of lesions at this site between test and reference cigarette smoke-exposed groups did not indicate any statistically significant differences in either study. Minimal or mild squamous metaplasia of the mucosal epithelium lining the caudal larynx was observed in 2/20 rats exposed to the 0.8 mg/L concentration of smoke from the test cigarette and 1/20 rats exposed to the 0.8 mg/L concentration of smoke from the reference cigarette in study 1.

Exposure to smoke from reference or test cigarettes induced a dose-related increase in minimal hyperplasia of the mucosal epithelium lining the tracheal lumen in both sexes of rats in study 1 and in males in study 2. Comparison of incidence in groups exposed to similar concentrations of smoke from test and reference cigarettes did not indicate any statistical differences in either study.

There were increased numbers of macrophages diffusely scattered through the pulmonary alveoli of rats exposed to smoke from reference or test cigarettes in both studies, compared to concurrent controls. There was some evidence of a dose response in the incidence and severity of macrophage accumulation in alveoli of smoke-exposed rats. This increase was graded as minimal in the vast majority of affected rats. Comparison of incidence and severity data for macrophages in alveoli of rats exposed to smoke from the test and reference cigarettes did not indicate any statistically significant differences. Minimal goblet-cell hyperplasia was observed in AB/PAS-stained sections of the mainstem bronchi of some rats exposed to smoke from reference or test cigarettes in both studies. There was some evidence of a dose response in the incidence of this lesion. Analysis of data indicated a statistically significant increase compared to controls in rats of both sexes exposed to the 0.8 mg/L concentration of smoke from reference cigarettes and in female rats exposed to the 0.8-mg/L concentration of smoke from the test cigarette in study 1, and in both sexes exposed to 0.8 mg/L of reference cigarette smoke in study 2. The incidence (7/20) of goblet-cell hyperplasia in males exposed to the 0.8-mg/L concentration of smoke from the test cigarette in both studies, although not statistically significant, was considered to be toxicologically significant. The incidence of bronchial goblet-cell hyperplasia was slightly higher in male rats exposed to smoke from reference cigarettes compared to similar concentrations of smoke from test cigarettes, but comparison of incidence in groups exposed to similar concentrations of smoke from test and reference cigarettes did not indicate any statistical differences. There was a very low incidence of a variety of microscopic lesions in other tissues examined in both studies, with no evidence of an effect of exposure to smoke from the reference or test cigarette on these tissues.

TABLE 9
Study 1, summary of microscopic observations with average severity in rats

Organ/diagnosis	Incidence of lesions (mean severity, if applicable) by target exposure concentration (mg WTPM/L)						
		Test			Reference		
	Sham controls	0.06	0.2	0.8	0.06	0.2	0.8
				Males			
Nose/turbinates	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]
Respiratory epithelium, hyperplasia	0[b] (0.0)	2 (0.2)	4 (0.3)	20 (2.2)	1 (0.1)	8 (0.4)	20 (2.1)
Goblet-cell hyperplasia	2 (0.1)	6 (0.3)	3 (0.2)	9 (0.5)	5 (0.3)	5 (0.3)	10 (0.5)
Suppurative inflammation	2 (0.2)	2 (0.3)	0 (0.0)	1 (0.1)	0 (0.0)	0 (0.0)	1 (0.1)
Larynx	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]
Epiglottis, squamous metaplasia	0 (0.0)	20 (2.2)	20 (2.9)	20 (3.0)	20 (2.1)	20 (2.9)	20 (3.1)
Epiglottis, epithelial hyperplasia	0 (0.0)	20 (2.2)	20 (2.9)	20 (3.0)	20 (2.1)	20 (2.9)	20 (3.0)
Epiglottis, hyperkeratosis	0 (0.0)	9 (0.5)	20 (1.4)	19 (1.9)	16 (0.9)	20 (1.8)	20 (1.9)
Ventral pouch, squamous metaplasia	0 (0.0)	12 (0.7)	20 (2.4)	20 (2.8)	7 (0.5)	19 (2.7)	20 (2.9)
Ventral pouch, epithelial hyperplasia	0 (0.0)	12 (0.7)	20 (2.4)	20 (2.8)	7 (0.5)	19 (2.7)	20 (2.9)
Ventral pouch, hyperkeratosis	0 (0.0)	0 (0.0)	9 (0.6)	19 (1.4)	1 (0.2)	17 (1.4)	18 (1.5)
Chronic inflammation	0 (0.0)	2 (0.1)	8 (0.4)	16 (0.9)	0 (0.0)	4 (0.2)	13 (0.7)
Caudal larynx, squamous metaplasia	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)
Trachea	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]
Epithelial hyperplasia	1 (0.1)	6 (0.3)	6 (0.3)	18 (0.9)	5 (0.3)	12 (0.6)	16 (0.8)
Lung	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]
Alveoli, macrophages	3 (0.2)	15 (0.8)	14 (0.7)	20 (1.4)	8 (0.4)	11 (0.6)	20 (1.1)
Bronchi, goblet-cell hyperplasia	0 (0.0)	1 (0.1)	1 (0.1)	7 (0.4)	3 (0.2)	4 (0.2)	11 (0.6)
Alveoli, hemorrhage	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.1)	0 (0.0)
				Females			
Nose/turbinates	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]
Respiratory epithelium, hyperplasia	0[b] (0.0)	0 (0.0)	7 (0.4)	20 (2.0)	0 (0.0)	3 (0.2)	20 (2.1)
Goblet-cell hyperplasia	2 (0.1)	2 (0.1)	2 (0.1)	7 (0.4)	2 (0.1)	2 (0.1)	4 (0.2)
Suppurative inflammation	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Larynx	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]
Epiglottis, squamous metaplasia	0 (0.0)	20 (2.2)	20 (3.0)	20 (3.1)	20 (2.2)	20 (2.6)	20 (3.1)
Epiglottis, epithelial hyperplasia	0 (0.0)	20 (2.2)	20 (3.0)	20 (3.1)	20 (2.2)	20 (2.6)	20 (3.0)
Epiglottis, hyperkeratosis	0 (0.0)	19 (1.4)[c]	20 (2.2)	20 (2.2)	13 (0.7)	20 (2.0)	20 (2.1)
Ventral pouch, squamous metaplasia	0 (0.0)	10 (0.6)	20 (2.7)	20 (3.0)	12 (0.8)	20 (2.7)	20 (2.9)
Ventral pouch, epithelial hyperplasia	0 (0.0)	10 (0.6)	20 (2.7)	20 (3.0)	12 (0.8)	20 (2.7)	20 (2.9)
Ventral pouch, hyperkeratosis	0 (0.0)	0 (0.0)	15 (1.3)	20 (1.8)	1 (0.1)	18 (1.5)	18 (1.5)
Chronic inflammation	0 (0.0)	3 (0.2)	2 (0.2)	10 (0.6)	0 (0.0)	4 (0.2)	17 (1.0)
Caudal larynx, squamous metaplasia	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.1)	0 (0.0)	0 (0.0)	1 (0.1)
Trachea	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]
Epithelial hyperplasia	1 (0.1)	2 (0.1)	8 (0.4)	12 (0.6)	3 (0.2)	7 (0.4)	18 (0.9)
Lung	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]
Alveoli, macrophages	3 (0.2)	10 (0.5)	13 (0.7)	20 (1.2)	12 (0.6)	17 (0.9)	20 (1.3)
Bronchi, goblet-cell hyperplasia	0 (0.0)	2 (0.1)	3 (0.2)	10 (0.5)	1 (0.1)	4 (0.2)	13 (0.7)
Alveoli, hemorrhage	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

Note. Severity: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked.
[a]Number of tissues or animals examined.
[b]Number of diagnoses made.
[c]$p < .05$, Kolmogorov–Smirnov test, compared to 0.06-mg/L reference group.

TABLE 10
Study 2, summary of microscopic observations with average severity in rats

Organ/diagnosis	Incidence of lesions (mean severity, if applicable) by target exposure concentration (mg WTPM/L)						
		Test			Reference		
	Sham controls	0.06	0.2	0.8	0.06	0.2	0.8
				Males			
Nose/turbinates	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]
Respiratory epithelium, hyperplasia	0[b] (0.0)	0 (0.0)	2 (0.1)	20 (2.0)	0 (0.0)	4 (0.2)	20 (1.9)
Goblet-cell hyperplasia	2 (0.1)	3 (0.2)	3 (0.2)	3 (0.2)	3 (0.2)	4 (0.2)	3 (0.2)
Suppurative inflammation	0 (0.0)	2 (0.2)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.1)	0 (0.0)
Larynx	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]
Epiglottis, squamous metaplasia	0 (0.0)	20 (1.8)	20 (2.4)	20 (3.0)	20 (1.9)	20 (2.5)	20 (3.0)
Epiglottis, epithelial hyperplasia	0 (0.0)	20 (1.8)	20 (2.4)	20 (3.0)	20 (1.9)	20 (2.5)	20 (3.0)
Epiglottis, hyperkeratosis	0 (0.0)	6 (0.4)	15 (1.2)	20 (2.0)	13 (1.0)	20 (1.8)	20 (2.1)
Ventral pouch, squamous metaplasia	0 (0.0)	1 (0.1)	18 (1.4)	20 (1.8)	1 (0.1)	16 (1.2)	20 (1.8)
Ventral pouch, epithelial hyperplasia	0 (0.0)	1 (0.1)	18 (1.4)	20 (1.8)	1 (0.1)	16 (1.2)	20 (1.8)
Ventral pouch, hyperkeratosis	0 (0.0)	0 (0.0)	6 (0.4)	16 (1.2)	0 (0.0)	5 (0.4)	16 (1.0)
Trachea	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]
Epithelial hyperplasia	2 (0.1)	8 (0.4)	9 (0.5)	11 (0.6)	6 (0.3)	8 (0.4)	10 (0.5)
Lung	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]
Alveoli, macrophages	4 (0.2)	11 (0.6)	16 (0.9)	20 (1.4)	11 (0.6)	14 (0.7)	20 (1.4)
Alveoli, hemorrhage	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)
Chronic inflammation	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Bronchi, goblet-cell hyperplasia	0 (0.0)	1 (0.1)	1 (0.1)	4 (0.2)	0 (0.0)	1 (0.1)	9 (0.5)
				Females			
Nose/turbinates	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]
Respiratory epithelium, hyperplasia	0[b] (0.0)	0 (0.0)	4 (0.2)	20 (1.5)	0 (0.0)	4 (0.2)	20 (1.6)
Goblet-cell hyperplasia	3 (0.2)	3 (0.2)	5 (0.3)	5 (0.3)	5 (0.3)	2 (0.1)	8 (0.4)
Suppurative inflammation	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.1)	0 (0.0)	0 (0.0)
Larynx	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]
Epiglottis, squamous metaplasia	0 (0.0)	20 (1.9)	20 (2.8)	20 (2.8)	20 (1.8)	20 (2.6)	20 (2.6)
Epiglottis, epithelial hyperplasia	0 (0.0)	20 (1.9)	20 (2.8)	20 (2.8)	20 (1.8)	20 (2.6)	20 (2.6)
Epiglottis, hyperkeratosis	0 (0.0)	16 (1.0)	20 (2.0)	20 (2.2)	15 (0.9)	20 (1.6)	20 (2.4)
Ventral pouch, squamous metaplasia	0 (0.0)	1 (0.1)	15 (1.2)	19 (1.9)	2 (0.1)	16 (1.1)	20 (2.0)
Ventral pouch, epithelial hyperplasia	0 (0.0)	1 (0.1)	14 (1.1)	19 (1.9)	2 (0.1)	16 (1.1)	20 (2.0)
Ventral pouch, hyperkeratosis	0 (0.0)	0 (0.0)	6 (0.5)	18 (1.4)	0 (0.0)	9 (0.6)	20 (1.7)
Trachea	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]
Epithelial hyperplasia	1 (0.1)	0 (0.0)	1 (0.1)	2 (0.1)	2 (0.1)	1 (0.1)	2 (0.1)
Lung	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]
Alveoli, macrophages	3 (0.2)	9 (0.5)	10 (0.5)	19 (1.1)	10 (0.5)	10 (0.5)	17 (1.0)
Perivascular lymphoid infiltrate	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)
Alveoli, hemorrhage	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Chronic inflammation	0 (0.0)	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Bronchi, goblet-cell hyperplasia	0 (0.0)	1 (0.1)	0 (0.0)	7 (0.4)	3 (0.2)	4 (0.2)	10 (0.5)

Note. Severity: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked.
[a]Number of tissues or animals examined.
[b]Number of diagnoses made.

Examination of tissue sections from rats necropsied at the end of the recovery period demonstrated nearly complete regression of nasal and tracheal lesions and a substantial decrease in the incidence and severity of smoke-induced lesions in the larynx and lungs in rats exposed to smoke from test or reference cigarettes in both studies. Macrophages observed in alveoli of smoke-exposed and control recovery group rats were in small focal aggregates, as opposed to the diffuse distribution of macrophages in lungs of rats necropsied at the interim sacrifice. There was no statistically significant difference in the incidence or severity of respiratory-tract lesions between recovery group rats previously exposed to similar concentrations of test and reference cigarette smoke in either study.

Evaluation of Cell Proliferation Rates

There was a dose-related trend toward higher mean nuclear labeling rates in the epithelium lining the median nasal septum in groups exposed to progressively higher concentrations of test or reference cigarette smoke compared to sham controls, but the increases were statistically significant only in females exposed to 0.8 mg/L of test cigarette smoke in study 1 and males exposed to 0.8 mg/L of reference cigarette smoke in study 2. Mean nuclear labeling rates of nasal epithelium lining the distal portions of the nasal and maxillary turbinates were statistically increased compared to control rates in both sexes of rats exposed to 0.8 mg/L of smoke from the test or reference cigarettes in both studies. Mean labeling rates in nasal and maxillary turbinates of study 1 males exposed to 0.8 mg/L of test cigarette smoke were statistically increased compared to labeling rates at these sites in males exposed to the same concentration of reference cigarette smoke.

Mean nuclear labeling rates in laryngeal epithelium were increased compared to sham control groups at all dose levels in both studies. Labeling rates in laryngeal epithelium were statistically different between several test and reference cigarette smoke-exposed groups in both studies, with no clear trend. The histopathology findings of laryngeal epithelial hyperplasia in smoke-exposed rats confirmed the relative sensitivity of these laryngeal sites to smoke-induced hyperplastic changes.

Mean nuclear labeling rates in the tracheal epithelium of rats exposed to smoke from test or reference cigarettes were not clearly different from those of sham controls of the same sex in either study. Labeling rates of bronchial, bronchiolar, and alveolar epithelium in both studies were difficult to evaluate due to wide standard deviations, low labeling rates, and variable sample sizes, and therefore labeling data from these sites were not used in evaluating effects of smoke exposure.

DISCUSSION

The studies described here were designed to evaluate the potential influence of ingredients on the chemical composition and the biological activity of mainstream cigarette smoke. Test cigarettes containing flavorings or casings were analyzed and compared against reference cigarettes identical except produced without flavors or casings. The configuration and ISO-condition tar, nicotine, and CO yields of all cigarettes investigated are representative of American blend cigarettes. Both test and reference cigarettes had the same tobacco blend and humectant composition (glycerine plus water) and were prepared by the same manufacturing process. Similarly, identical nontobacco materials (NTM) were used throughout. The weight of the filler remained constant between test and reference cigarettes. These studies illustrate that the application of 165 low-use flavoring or 8 high-use flavoring or casing ingredients had little, if any, observable effect on the deliveries or physical parameters of the cigarettes.

From comparison of the mutagenicity data obtained in Ames assays of studies 1 and 2 test and reference cigarettes, it was concluded that the addition of these ingredients did not increase the mutagenic response of any of the strains of *Salmonella typhimurium* under the conditions described, and the results did not suggest any mutagenic activity of the added ingredients.

The objectives of the two inhalation toxicity studies were to compare the biologic activity of mainstream smoke from the two test cigarettes with reference cigarettes in a series of two 13-wk inhalation exposures, each followed by a 13-wk recovery period. Data collected during the 13-wk exposures confirmed that both the particulate (WTPM, nicotine) and vapor (CO) phases of the inhalation atmospheres presented to the rats were well controlled and provided appropriate data for comparison of the responses of the study animals to smoke from the two cigarettes under investigation in each of the two studies. WTPM was used as the basis for exposure concentration in these studies, since the predominant known toxicologic effects of cigarette smoke are associated with the mainstream particulate phase (Coggins et al., 1980).

Blood COHb concentrations demonstrated that exposure of rats to smoke from either the test or reference cigarette resulted in reproducible biomarkers of exposure consistent with the concentration of CO in the smoke. Samples taken for plasma nicotine analysis confirmed exposure to nicotine in test or reference smoke, which resulted in exposure-related increases in plasma nicotine concentrations.

The only occurrence during either study that affected the utility of the data was the failure to fast the sham control rats prior to necropsy at the interim sacrifice immediately following the exposure period in study 2. This error did not allow direct comparison of the body and organ weights of controls with smoke-exposed groups sacrificed at that time point.

Other investigations have noted effects similar to those we observed of cigarette smoke exposure on body weight, including the relative resistance of females to this change (Coggins et al., 1989; Baker et al., 2004). We concluded that the decreased body weights in smoke-exposed groups in both studies compared to sham controls were the result of smoke exposure. However, we do not consider these effects on body weight to be toxicologically significant due to their recovery after smoke exposure was terminated, and due to the lack of any concurrent clinical observations that would indicate any significant dysfunction.

In study 1 there were a number of statistically significant differences in absolute or relative organ weights between test or reference cigarette smoke-exposed groups and sham controls necropsied immediately following 13 wk of smoke exposure. However, these statistical differences showed no clear dose-response pattern, and no exposure-related histopathologic effects were observed in any weighed organ except the lungs. It is possible that the increased lung/body weight ratios in study 1 rats exposed to 0.8-mg/L of smoke from test or reference cigarettes were related to the minimal increase in numbers of macrophages in alveoli of these rats. These increases in lung/body weight ratio more likely reflect the decreased body weight in these groups at the interim sacrifice. In any case, these and the other statistical differences in absolute or relative organ weights in smoke-exposed rats compared to sham controls are not considered toxicologically significant. There was no consistent difference in organ weights between groups of rats exposed to similar concentrations of test and reference cigarette smoke in either study. Increases in total inhaled mass were proportional to increasing exposure concentration in study 1, but in study 2 decreases in MV in groups exposed to 0.8- or 0.2-mg/L relative to groups exposed to 0.06 mg/L caused total inhaled mass for the high and middle dose groups to be lower in proportion to exposure concentration of smoke.

Inhalation exposure to smoke from test or reference cigarettes in both studies clearly induced microscopic changes in the nasal cavity, larynx, trachea, and lungs of exposed rats. Results of histopathologic examination of the recovery groups illustrated that these respiratory-tract lesions were either completely resolved or in the process of resolving by 13 wk after cessation of smoke exposure, and thus represent an adaptive response to the inhaled smoke. The nasal cavity and larynx were much more affected by inhaled smoke than the lungs in our studies, and the mucosal epithelium lining the base of the epiglottis and adjacent ventral pouch was the most affected site. The extreme susceptibility of the rodent laryngeal mucosa to inhaled smoke and other xenobiotics has been described in detail (Lewis, 1980, 1991; Gopinath et al., 1987; Burger et al., 1989). Since the most notable cellular changes observed in the respiratory tract of rodents in response to inhaled smoke involve cellular proliferation and metaplasia, a quantitative measure of cell turnover in affected tissue is a useful tool to measure the effect of exposure. Cell proliferation rate measurements in nasal turbinates and laryngeal epithelium using nuclear labeling with BrdU correlated well with histopathology data, reinforcing the conclusion that exposure to smoke from test or reference cigarette smoke for 13 wk clearly induced epithelial hyperplasia at these sites. Results of BrdU labeling in the trachea and lungs were less clear, and probably reflect the more subtle effects of inhaled smoke on the epithelium at these sites.

The effects of inhaled cigarette smoke on the respiratory tract of rats in both the studies described herein are similar to those described in a number of previously reported cigarette smoke inhalation studies in rats (Dalbey et al., 1980; Gaworski et al., 1997; Coggins et al., 1989; Ayres et al., 2001; Vanscheeuwijck et al., 2002) and hamsters (Lewis, 1980; Wehner et al., 1990). Four recently published papers have described studies similar to those presented here, in which smokes from cigarettes with and without flavoring or casing ingredients were compared on the basis of chemical composition and biologic effects on rodents (Gaworski et al., 1998; Paschke et al., 2002; Carmines, 2002; Baker et al., 2004). Results of the studies presented here are consistent with the conclusions of these authors that the presence of flavoring and casing ingredients studied to date did not significantly change the type or extent of toxicologic effects observed in rodents inhaling cigarette smoke.

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SAFETY DATA SHEET

according to Regulation (EC) No. 1907/2006

Version 6.3
Revision Date 15.09.2021
Print Date 08.10.2022

SECTION 1: Identification of the substance/mixture and of the company/undertaking

1.1 Product identifiers

Product name : Ethyl butyrate

Product Number : E15701
Brand : Aldrich
REACH No. : 01-2120118576-54-XXXX
CAS-No. : 105-54-4

1.2 Relevant identified uses of the substance or mixture and uses advised against

Identified uses : Laboratory chemicals, Manufacture of substances

1.3 Details of the supplier of the safety data sheet

Company : Merck Life Science UK Limited
New Road
The Old Brickyard
GILLINGHAM
Dorset
SP8 4XT
UNITED KINGDOM

Telephone : +44 (0)1747 833-000
Fax : +44 (0)1747 833-313
E-mail address : TechnicalService@merckgroup.com

1.4 Emergency telephone

Emergency Phone # : +44 (0)870 8200418 (CHEMTREC)

SECTION 2: Hazards identification

2.1 Classification of the substance or mixture

Classification according to Regulation (EC) No 1272/2008
Flammable liquids (Category 3), H226

For the full text of the H-Statements mentioned in this Section, see Section 16.

2.2 Label elements

Labelling according Regulation (EC) No 1272/2008
Pictogram

Signal word Warning

Hazard statement(s) H226	Flammable liquid and vapor.
Precautionary statement(s) P210	Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
Supplemental Hazard Statements	none

Reduced Labeling (<= 125 ml)

Pictogram	
Signal word	Warning
Hazard statement(s)	none
Precautionary statement(s)	none
Supplemental Hazard Statements	none

2.3 Other hazards

This substance/mixture contains no components considered to be either persistent, bioaccumulative and toxic (PBT), or very persistent and very bioaccumulative (vPvB) at levels of 0.1% or higher.

SECTION 3: Composition/information on ingredients

3.1 Substances

Synonyms	:	Butyric acid ethyl ester
Formula	:	$C_6H_{12}O_2$
Molecular weight	:	116.16 g/mol
CAS-No.	:	105-54-4
EC-No.	:	203-306-4

Component		Classification	Concentration
Ethyl butyrate			
CAS-No. EC-No.	105-54-4 203-306-4	Flam. Liq. 3; H226	<= 100 %

For the full text of the H-Statements mentioned in this Section, see Section 16.

SECTION 4: First aid measures

4.1 Description of first-aid measures

General advice

Consult a physician. Show this material safety data sheet to the doctor in attendance.

If inhaled

If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician.

In case of skin contact
Wash off with soap and plenty of water. Consult a physician.

In case of eye contact
Rinse thoroughly with plenty of water for at least 15 minutes and consult a physician.

If swallowed
Do NOT induce vomiting. Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.

4.2 Most important symptoms and effects, both acute and delayed
The most important known symptoms and effects are described in the labelling (see section 2.2) and/or in section 11

4.3 Indication of any immediate medical attention and special treatment needed
No data available

SECTION 5: Firefighting measures

5.1 Extinguishing media

Suitable extinguishing media
Dry powder Dry sand

Unsuitable extinguishing media
Do NOT use water jet.

5.2 Special hazards arising from the substance or mixture
Carbon oxides
Combustible.

5.3 Advice for firefighters
Wear self-contained breathing apparatus for firefighting if necessary.

5.4 Further information
Use water spray to cool unopened containers.

SECTION 6: Accidental release measures

6.1 Personal precautions, protective equipment and emergency procedures
Use personal protective equipment. Avoid breathing vapors, mist or gas. Ensure adequate ventilation. Remove all sources of ignition. Beware of vapors accumulating to form explosive concentrations. Vapors can accumulate in low areas.
For personal protection see section 8.

6.2 Environmental precautions
Prevent further leakage or spillage if safe to do so. Do not let product enter drains.

6.3 Methods and materials for containment and cleaning up
Contain spillage, and then collect with non-combustible absorbent material, (e.g. sand, earth, diatomaceous earth, vermiculite) and place in container for disposal according to local / national regulations (see section 13).

6.4 Reference to other sections
For disposal see section 13.

SECTION 7: Handling and storage

7.1 Precautions for safe handling

Advice on safe handling
Avoid contact with skin and eyes. Avoid inhalation of vapor or mist.

Advice on protection against fire and explosion
Keep away from sources of ignition - No smoking.Take measures to prevent the build up of electrostatic charge.

Hygiene measures
Handle in accordance with good industrial hygiene and safety practice. Wash hands before breaks and at the end of workday.
For precautions see section 2.2.

7.2 Conditions for safe storage, including any incompatibilities

Storage conditions
Keep container tightly closed in a dry and well-ventilated place. Containers which are opened must be carefully resealed and kept upright to prevent leakage. Store in cool place.

Storage class
Storage class (TRGS 510): 3: Flammable liquids

7.3 Specific end use(s)

Apart from the uses mentioned in section 1.2 no other specific uses are stipulated

SECTION 8: Exposure controls/personal protection

8.1 Control parameters

Ingredients with workplace control parameters
Contains no substances with occupational exposure limit values.

8.2 Exposure controls

Personal protective equipment

Eye/face protection
Face shield and safety glasses Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU).

Skin protection
Handle with gloves. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands.

The selected protective gloves have to satisfy the specifications of Regulation (EU) 2016/425 and the standard EN 374 derived from it.

Splash contact
Material: butyl-rubber
Minimum layer thickness: 0.3 mm
Break through time: 45 min
Material tested:Butoject® (KCL 897 / Aldrich Z677647, Size M)

data source: KCL GmbH, D-36124 Eichenzell, phone +49 (0)6659 87300, e-mail sales@kcl.de, test method: EN374
If used in solution, or mixed with other substances, and under conditions which differ from EN 374, contact the supplier of the EC approved gloves. This recommendation is advisory only and must be evaluated by an industrial hygienist and safety officer familiar with the specific situation of anticipated use by our customers. It should not be construed as offering an approval for any specific use scenario.

Body Protection
Impervious clothing, Flame retardant antistatic protective clothing., The type of protective equipment must be selected according to the concentration and amount of the dangerous substance at the specific workplace.

Respiratory protection
Where risk assessment shows air-purifying respirators are appropriate use a full-face respirator with multi-purpose combination (US) or type ABEK (EN 14387) respirator cartridges as a backup to engineering controls. If the respirator is the sole means of protection, use a full-face supplied air respirator. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

Control of environmental exposure
Prevent further leakage or spillage if safe to do so. Do not let product enter drains.

SECTION 9: Physical and chemical properties

9.1 Information on basic physical and chemical properties

a)	Appearance	Form: liquid Color: colorless
b)	Odor	fruity
c)	Odor Threshold	No data available
d)	pH	No data available
e)	Melting point/freezing point	Melting point: -98 °C
f)	Initial boiling point and boiling range	121 °C at 972 hPa - OECD Test Guideline 103
g)	Flash point	26 °C - c.c.
h)	Evaporation rate	No data available
i)	Flammability (solid, gas)	No data available
j)	Upper/lower flammability or explosive limits	No data available
k)	Vapor pressure	17 hPa at 20 °C
l)	Vapor density	No data available
m)	Density	0.87 g/cm3 at 20 °C - OECD Test Guideline 109
	Relative density	No data available
n)	Water solubility	0.21 g/l at 30 °C

o)	Partition coefficient: n-octanol/water	log Pow: 2.85 at 25 °C
p)	Autoignition temperature	463 °C
q)	Decomposition temperature	No data available
r)	Viscosity	Viscosity, kinematic: No data available Viscosity, dynamic: No data available
s)	Explosive properties	No data available
t)	Oxidizing properties	none

9.2 Other safety information
No data available

SECTION 10: Stability and reactivity

10.1 Reactivity
No data available

10.2 Chemical stability
Stable under recommended storage conditions.

10.3 Possibility of hazardous reactions
No data available

10.4 Conditions to avoid
Heat, flames and sparks.

10.5 Incompatible materials
No data available

10.6 Hazardous decomposition products
In the event of fire: see section 5

SECTION 11: Toxicological information

11.1 Information on toxicological effects

Acute toxicity
LD50 Oral - Rat - female - > 2,000 mg/kg
(OECD Test Guideline 423)
Symptoms: Nausea, Vomiting, Risk of aspiration upon vomiting., Aspiration may cause pulmonary edema and pneumonitis.
Inhalation: No data available
LD50 Dermal - Rat - male and female - > 2,000 mg/kg
(OECD Test Guideline 402)

Skin corrosion/irritation
Skin - Rat
Result: No skin irritation - 24 h
(Patch Test 24 Hrs.)

Serious eye damage/eye irritation
Eyes - Rabbit
Result: No eye irritation
Remarks: (ECHA)

Respiratory or skin sensitization
Open epicutaneous test - Guinea pig
Result: negative
Remarks: (ECHA)

Germ cell mutagenicity
Test Type: Ames test
Test system: Salmonella typhimurium
Metabolic activation: with and without metabolic activation
Method: OECD Test Guideline 471
Result: negative

Carcinogenicity
No data available

Reproductive toxicity
No data available

Specific target organ toxicity - single exposure
No data available

Specific target organ toxicity - repeated exposure
No data available

Aspiration hazard
No data available

11.2 Additional Information

RTECS: ET1660000
To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

If inhaled

After uptake of large quantities:

Possible symptoms:

Dizziness
somnolence
Unconsciousness
CNS disorders

Other dangerous properties can not be excluded.

Handle in accordance with good industrial hygiene and safety practice.

SECTION 12: Ecological information

12.1 Toxicity

Toxicity to fish	LC50 - Leuciscus idus (Golden orfe) - 53 mg/l - 48 h Remarks: (ECOTOX Database)
Toxicity to daphnia and other aquatic invertebrates	static test EC50 - Daphnia magna (Water flea) - 116.6 mg/l - 48 h (OECD Test Guideline 202)

Toxicity to algae	static test ErC50 - Desmodesmus subspicatus (green algae) - > 100 mg/l - 72 h (OECD Test Guideline 201)
Toxicity to bacteria	EC50 - activated sludge - 6,070 mg/l (OECD Test Guideline 209)

12.2 Persistence and degradability

Biodegradability	Result: 63 % - Readily biodegradable. (OECD Test Guideline 301F)

12.3 Bioaccumulative potential

No data available

12.4 Mobility in soil

No data available

12.5 Results of PBT and vPvB assessment

This substance/mixture contains no components considered to be either persistent, bioaccumulative and toxic (PBT), or very persistent and very bioaccumulative (vPvB) at levels of 0.1% or higher.

12.6 Other adverse effects

Discharge into the environment must be avoided.

SECTION 13: Disposal considerations

13.1 Waste treatment methods

Product

Offer surplus and non-recyclable solutions to a licensed disposal company. Waste material must be disposed of in accordance with the Directive on waste 2008/98/EC as well as other national and local regulations. Leave chemicals in original containers. No mixing with other waste. Handle uncleaned containers like the product itself.

Contaminated packaging

Dispose of as unused product.

SECTION 14: Transport information

14.1 UN number

ADR/RID: 1180	IMDG: 1180	IATA: 1180

14.2 UN proper shipping name

ADR/RID:	ETHYL BUTYRATE
IMDG:	ETHYL BUTYRATE
IATA:	Ethyl butyrate

14.3 Transport hazard class(es)

ADR/RID: 3	IMDG: 3	IATA: 3

14.4 Packaging group

ADR/RID: III	IMDG: III	IATA: III

14.5 Environmental hazards

ADR/RID: no	IMDG Marine pollutant: no	IATA: no

14.6 Special precautions for user

No data available

SECTION 15: Regulatory information

15.1 Safety, health and environmental regulations/legislation specific for the substance or mixture

This material safety data sheet complies with the requirements of Regulation (EC) No. 1907/2006.

National legislation

Seveso III: Directive 2012/18/EU of the European Parliament and of the Council on the control of major-accident hazards involving dangerous substances.	: FLAMMABLE LIQUIDS

15.2 Chemical Safety Assessment

For this product a chemical safety assessment was not carried out

SECTION 16: Other information

Full text of H-Statements referred to under sections 2 and 3.

H226	Flammable liquid and vapor.

Further information

The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. The information in this document is based on the present state of our knowledge and is applicable to the product with regard to appropriate safety precautions. It does not represent any guarantee of the properties of the product. Sigma-Aldrich Corporation and its Affiliates shall not be held liable for any damage resulting from handling or from contact with the above product. See www.sigma-aldrich.com and/or the reverse side of invoice or packing slip for additional terms and conditions of sale.

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