Toxicological profile for

Sugars (invert sugar)

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

2,3,4,5,6-Pentahydroxyhexanal;1,3,4,5,6-pentahydroxyhexan-2-one and (2R,3S,4R,5R)-2,3,4,5,6-pentahydroxyhexanal;(3S,4R,5R)-1,3,4,5,6-pentahydroxyhexan-2-one (PubChem)

1.2. Synonyms

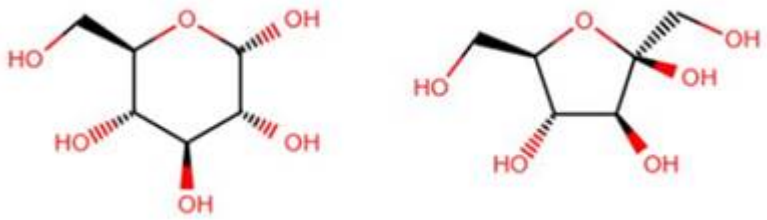
Calorose; EINECS 232-393-1; HS 500; HSDB 2008; Insubeta; Inverdex; Invert sugar; Invertix; Invertogen; Invertose; Lumolinine; Metabol; Nevuline; Nulomoline; Sugar, invert; Travert; Trimolin; UNII-ED959S6ACY (ChemIDplus)

1.3. Molecular formula

C12 H24 O12 (PubChem); “unspecified” (ChemIDplus)

1.4. Structural Formula

Mixture of approximately 50% dextrose and 50% levulose obtained by hydrolysis of cane sugar (ChemIDplus)



“Saccharide hydrolysate is an invert sugar derived by the hydrolysis of sucrose by acid, enzyme, or other method of hydrolysis. It is characterized by a content of fructose and glucose" (CIR, 2020).

1.5. Molecular weight (g/mol)

360.3096 (ChemIDplus)

1.6. CAS registration number

8013-17-0

1.7. Properties

1.7.1. Melting point

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

1.7.2. Boiling point

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

1.7.3. Solubility

Very soluble in water (CIR, 2020)

1.7.4. pKa

No data available to us at this time.

1.7.5. Flashpoint

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

1.7.6. Flammability limits (vol/vol%)

No data available to us at this time.

1.7.7. (Auto)ignition temperature

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

1.7.8. Decomposition temperature

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

1.7.9. Stability

No data available to us at this time.

1.7.10. Vapor pressure

No data available to us at this time.

1.7.11. log Kow

-1.46 or -2.43 (estimated) (CIR, 2020)

2. General information

2.1. Exposure

“Cane juice contains ca 5-10 wt% invert sugar (based on sucrose), whereas in healthy beet sugar it is 1%”

As taken from HSDB, 2002.

In Europe, saccharide hydrolysate (CAS RN 8013-17-0) is used as a humectant in cosmetics. As taken from Cosing.

Invert sugar (CAS RN 8013-17-0) is listed as an intravenous antivaricose therapy on the WHO ATC/DDD (Anatomical Therapeutic Chemical classification index / Definition of Defined Dose) index (WHO, 2020).

Sugar, invert (CAS RN 8013-17-0) is listed as a fragrance ingredient by IFRA.

Sugar, invert “is used in the following products: cosmetics and personal care products, biocides (e.g. disinfectants, pest control products), fertilisers, leather treatment products, perfumes and fragrances, textile treatment products and dyes, fuels, laboratory chemicals, paper chemicals and

dyes, polishes and waxes and washing & cleaning products". As taken from ECHA Substance Infocard. Available at:https://echa.europa.eu/information-on-chemicals

Invert sugar (CAS RN 8013-17-0) is used as a sweetening agent in non-medicinal natural health products (Health Canada, 2022).

Saccharide hydrolysate (CAS RN 8013-17-0) is used as a skin protectant; skin conditioning agents and humectant.

"The results of a concentration of use survey conducted by the Council in 2018 indicate that Saccharide Hydrolysate [CAS RN 8013-17-0] is used at maximum use concentrations up to 4.6% in rinse off products (skin cleansing products)."

As taken from CIR, 2020

2.2. Combustion products

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

	Two stage heating		One stage heating	
Compound	Abundance	Area%	Abundance	Area%
acetic acid	15861208	1.98	24378307	3.58
unknown	3454437	0.42	7839692	1.15
furfural	103929069	12.95	93033460	13.65
5-methylfurfural	9701400	1.21	9279334	1.36
4,5-dimethylfurfural	10139731	1.26	8740637	1.28
2,3-dihydro-3,5-dihydroxmethyl-4H-pyran-4-one	11379290	1.42	18189498	2.67
1,4:3,6-dianhydro-alpha-D-lucopyranose	14393042	1.79	7844536	1.15
5-hydroxymethylfurfural	251053333	31.29	257452962	37.78
levoglucosan	201411147	25.10	119485007	17.53
unknown	1170764	0.14	13793462	2.02
1,6-anhydro-beta-D-glucofuranose	5977389	0.73	26713065	3.92
Total ion chromatogram	818820411	100	681455740	100

This ingredient was investigated in a pyrolysis study. Results are given in Baker and Bishop (2005) J. Anal. Appl. Pyrolysis 74, pp. 145–170.

Ingredient (maximum cigarette level, ppm)	Pyrolysis product (compound, %)	Maximum level in smoke from ingredient (ug/cigarette)	Typical smoke level (nü¡g/cigarette)
Sugar, invert (62,000)	Hydroxymethylfurfural 40.1	12000	
	Furfural 34.9	11000	15-43
	Glycoaldehyde and/or methyl formate 4.0	1200	
	Acetic acid 3.0	930	
	Pyruvaldehyde 2.3	710	

2.3. Ingredient(s) from which it originates

“Honey is mostly invert sugar”.

As taken from HSDB, 2002.

3. Status in legislation and other official guidance

FDA requirements:

PART 184-- Direct food substances affirmed as generally recognized as safe (GRAS).
Subpart B--Listing of Specific Substances Affirmed as GRAS
Sec. 184.1859 Invert sugar.
(a) Invert sugar (CAS Reg. No. 8013-17-0) is an aqueous solution of inverted or partly inverted, refined or partly refined sucrose, the solids of which contain not more than 0.3 percent by weight of ash. The solution is colorless, odorless, and flavorless, except for sweetness. It is produced by the hydrolysis or partial hydrolysis of sucrose with safe and suitable acids or enzymes.
(b) The ingredient must be of a purity suitable for its intended use.
(c) In accordance with § 184.1(b)(1), the ingredient is used in food with no limitation other than current good manufacturing practice.
(d) Prior sanctions for this ingredient different from the uses established in this section do not exist or have been waived. [53 FR 44876, Nov. 7, 1988; 54 FR 228, Jan. 4, 1989, as amended at 73 FR 8608, Feb. 14, 2008] .

As taken from FDA, 2022a

Invert sugar is listed in the US FDA’s Inventory of Substances Added to Food (formerly EAFUS) as a flavor enhancer, a flavoring agent or adjuvant, and a nutritive sweetener, and is also covered under 21 CFR sections 131.112 (cultured milk), 131.170 (eggnog), 131.200 (yogurt), 131.203 (low fat yogurt), 131.206 (nonfat yogurt), 146.140 (pasteurized orange juice), 146.141 (canned orange

juice), 146.145 (orange juice from concentrate), 146.146 (frozen concentrated orange juice), 169.175 (vanilla extract), 73.85 (caramel) (FDA, 2022a,b).

There is a REACH dossier on sugar, invert (ECHA, 2021).

Sugar, invert (CAS RN 8013-17-0) is not classified for packaging and labelling under Regulation (EC) No. 1272/2008 (ECHA, 2022).

Invert syrup (CAS RN 8013-17-0) is listed in the US EPA InertFinder Database (2022) as approved for food and non-food use pesticide products. For food use, it is regulated under 40 CFR Part 180.950a (Tolerances and Exemptions for Pesticide Chemical Residues in Food. Tolerance exemptions for minimal risk active and inert ingredients) (US EPA, 2022).

Invert sugar is listed in the US EPA Toxic Substances Control Act (TSCA) and 2020 CDR TSCA Inv Active inventories.

The TSCA inventory is available at https://sor.epa.gov/sor_internet/registry/substreg/LandingPage.do

Excipients and information for the package leaflet:

Name	Route of administration	Threshold	Information for the package leaflet	Comments
Invert sugar	Oral	Zero	If you have been told by your doctor that you have an intolerance to some sugars, contact your doctor before taking this medicinal product.	SmPC proposal: Patients with rare hereditary problems of fructose intolerance or glucose-galactose malabsorption should not take this medicine.
Invert sugar	Oral	5 g	Contains x g of a mixture of fructose and glucose per dose. This should be taken into account in patients with diabetes mellitus	
Invert sugar	Oral liquids, lozenges and chewable tablets	Zero	May be harmful to the teeth.	Information to be included only when the medicinal product may be intended for chronic use, e.g. for two weeks or more.

As taken from EMA, 2019.

Invert sugar (CAS RN 8013-17-0) “poses no unreasonable risk to human health based on Tier I assessment under the NICNAS IMAP assessment framework” and has been “identified as low concern to human health by application of expert validated rules under the NICNAS targeted tier I approach” (AICIS, 2017).

Invert sugar (CAS RN 8013-17-0) 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 potencies per unit dose:

Inactive Ingredient	**Route**	**Dosage Form**	**CAS Number**	**UNII**	**Maximum Potency per unit dose**
INVERT SUGAR	ORAL	CAPSULE	8013170	ED959S6ACY	NA

INVERT SUGAR	ORAL	SOLUTION	8013170	ED959S6ACY	3.06ml/5ml
INVERT SUGAR	ORAL	SYRUP	8013170	ED959S6ACY	2560mg/5ml

As taken from FDA, 2022c

4. Metabolism/Pharmacokinetics

4.1. Metabolism/metabolites

Demonstration of a specific metabolic effect of dietary disaccharides in the rat (Abstract). Male Wistar rats were starved and refed diets containing either 40% carbohydrate as monosaccharides (glucose, fructose, invert sugar) or disaccharides (maltose, sucrose), or 42.2% carbohydrate as glucose. Induction of various liver enzymes and changes in total liver lipid levels by the different dietary sugars were studied. Liver enzymes measured included glucose-6-phosphate dehydrogenase (g6pd), 6-phosphogluconate dehydrogenase (6PGD), malic enzyme (ME), phosphofructokinase (PFK), L-alpha-glycerol phosphate dehydrogenase (LalphaGPD), pyruvate kinase (PK), citrate cleavage enzyme (CCE), acetyl CoA carboxylase (AcCoAC), and fatty acid synthetase (FAS). The responses in enzyme activity to diets containing glucose or invert sugar were used as the basal response. Enzyme responses to refeeding the carbohydrate diets fell into three categories: (1) enzyme activity increased both by the disaccharide configuration of the carbohydrate and by fructose (G6PD, PK, CCE, AcCoAC, FAS); (2) enzyme activity increased only by the disaccharide configuration of the carbohydrate (6PGD, ME); and (3) enzyme activity increased only by fructose (PFK, LalphaGPD). Total liver lipid level was increased both by the disaccharide configuration of the carbohydrate and by fructose. Refeeding diets containing equal molar amounts of glucose or maltose did not abolish the disaccharide effect. The data indicate that the disaccharide configuration of maltose and sucrose may have an effect at the gastrointestinal level, which causes an increased induction of certain enzymes in the liver. As taken from Michaelis OE 4th; Nace CS; Szepesi B. J Nutr. 1975, Sep; 105(9):1186-91. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgicmd=retrieve&db=pubmed&list_uids=240012&dopt=AbstractPlus

4.2. Absorption, distribution and excretion

“Less than 2% of sugar is excreted in urine when 1 L of 10% invert sugar soln is infused in 1 hr. When given over a longer period of time invert sugar is completely utilited and none is excreted in urine.”
As taken from HSDB, 2002.

4.3. Interactions

”Chromium (III) (Cr(III)) effect on improving glucose, body mass loss, and genomic stability has been extensively studied in models of type 2 diabetes. However, there is a lack of studies evaluating its effect on prediabetes. Thus, this study evaluates the effects of Cr(III) as dietetic supplementation on glucose metabolism, obesity, and genomic stability on prediabetic rat model

using high-invert sugar. Male Wistar rats were divided randomly into four treatment groups: (1) control, receiving standard diet (control); (2) prediabetic (PD), receiving a 32% of invert sugar; (3) Cr(III), receiving chromium (III) chloride ($CrCl_3 \cdot 6H_2O$) (58.4 mg/L); and (4) Cr(III) + PD, receiving $CrCl_3 \cdot 6H_2O$ in combination with high-invert sugar. Cr(III) supplementation significantly reduced blood glucose (123.00 ± 8.29 mg/dL vs. 115.30 ± 9.31 mg/dL, $p = 0.015$) and partially reduced area under the 120-min blood glucose response curve (AUC) in PD rats ($p = 0.227$). Moreover, Cr(III) attenuated weight gain (187.29 ± 38.56 g vs. 167.22 ± 29.30 g, $p = 0.004$), significantly reducing body mass index (0.68 ± 0.04 g/cm2 vs. 0.63 ± 0.04 g/cm2, $p < 0.001$), Lee index (0.30 ± 0.01 vs. 0.28 ± 0.01, $p < 0.001$), and peritoneal fat ($p < 0.001$). Regarding genomic stability, high-invert sugar, Cr(III), or the combination of both did not produce changes in oxidative stress, DNA damage in pancreas, or cytotoxicity markers. These data suggest that Cr(III) supplementation improved partially glucose metabolism and reduced obesity in rat model PD due to high-invert sugar without influence in genomic stability." As taken from Molz P et al. 2021. Biol. Trace Elem. Res. 199(5), 1893-1899. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/32710349/

5. Toxicity

5.1. Single dose toxicity

No data available to us at this time.

5.2. Repeated dose toxicity

Quantitative Risk Type - Not calculated

Quantitative Risk Value - Not calculated

Product Use – Not specified

Safety Evaluation Owner - COSMOS TTC (NON-CANCER)

POD Method - LOAEL

POD Value – 3000.0

POD Owner - COSMOS TTC (NON-CANCER)

Adjustment factors used in calculations:

Adjustment factor: Study: Dose Duration: 3 (3);

Adjustment factor: Study: LOEL-NOEL Extrapolation: 3 (3)

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

NOEL/LEL Owner - PAFA

Original NOEL – Not established

Original LEL - 27000.0 mg/kg bw/day

Critical Effects - CLINICAL SIGNS - APPEARANCE/POSTURE

Safety Evaluation Comments: no comments available.

Source Document: no source document available

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

5.3. Reproduction toxicity

No data available to us at this time.

5.4. Mutagenicity

”The high consumption of sugars is linked to the intermediate hyperglycemia and impaired glucose tolerance associated with obesity, inducing the prediabetes. However, the consequences of excessive invert sugar intake on glucose metabolism and genomic stability were poorly studied. The aim of this study was to evaluate the effects of invert sugar overload (32%) in rats, analyzing changes in obesity, glucose tolerance, pancreatic/hepatic histology and primary and permanent DNA damage. After 17 weeks, the rats became obese and had an excessive abdominal fat, as well as presented impaired glucose tolerance, caused by higher sugar caloric intake. Primary DNA damage, evaluated by the comet assay, was increased in the blood, however not in the pancreas. No protein carbonylation was seen in serum. Moreover, no increase in permanent DNA damage was seen in the bone marrow, evaluated using the micronucleus test. Some rats presented liver steatosis and that the pancreatic islets were enlarged, but not significantly. In this study, invert sugar altered the glucose metabolism and induced primary DNA damage in blood, but did not cause significant damage to the pancreas or liver, and neither changes in the levels of oxidative stress or permanent DNA damage.” As taken from Molz P et al. 2020. An. Acad. Bras. Cienc. 92(2), e20191423. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/32696841/

5.5. Cytotoxicity

No data available to us at this time.

5.6. Carcinogenicity

No data available to us at this time.

5.7. Irritation/immunotoxicity

“Sc admin is not desirable because soln are irritating...cause leaching of extracellular water and electrolytes, and may distend tissue and lead to necrosis”

As taken from HSDB, 2002.

Sugar polymers in rats and man (Abstract). Intracutaneous injections of a glucan contaminant of invert sugar solutions and crude cane sugar into human skin produced a localized wheal and erythema reaction. The glucan was also active on intradermal injection into dextran-sensitive and dextran-resistant rats. As taken from Dhar HL ; Hanahoe T HP ; WEST GB INT ARCH ALLERGY APPL IMMUNOL; 51 (5). 1976 637-640. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/pubmed/965110

“A male patient had urticarial attacks over a period of 6 months after eating foods such as hamburgers, spaghetti, and cakes, and after consuming certain drinks. When the patient was given a refreshing drink (type not stated), urticarial lesions developed within 2 h. The ingredients of the drink were then given separately, with a week between each test. Two ingredients of the drink, invert sugar (also known as Saccharide Hydrolysate) and high-fructose corn syrup (containing mostly glucose and 0.07% Psicose), induced urticarial lesions. High-fructose corn syrup caused the stronger reaction, and a skin test on this ingredient (3 mg) yielded a positive reaction. Psicose was partly purified using thin layer chromatography, and yielded a positive skin reaction when applied at a dose of 21.8 µg. The authors concluded that Psicose was responsible for the urticarial attacks in the male patient.”

As taken from CIR, 2020

5.8. All other relevant types of toxicity

“Invert sugar injection (containing equal parts of dextrose and fructose): fructose offers no advantages and some disadvantages over dextrose injection. It may incr serum level of lactate and urate if given rapidly…infusion of fructose has been associated with incr production of uric acid and hyperuricemia.”

“Invert sugar injection (containing equal parts of dextrose and fructose): in pt with hereditary fructose intolerance (aldolase deficiency), fructose can cause severe reactions (hypoglycemia, nausea, vomiting, tremors, coma, convulsions) and is contraindicated.”

As taken from HSDB, 2002.

6. Functional effects on

6.1. Broncho/pulmonary system

No data available to us at this time.

6.2. Cardiovascular system

Effect of Intravenous Urea in Invert Sugar on Heme Catabolism in Sickle-Cell Anemia (Abstract).
Parenteral administration of urea in invert sugar, said to be effective in managing sickle-cell crises, may act in part by a hemolytic mechanism. Hence, we studied endogenous carbon monoxide production, an index of heme degradation, in five patients with sickle-cell anemia before and after the intravenous infusion of 80 to 90 g of urea in invert sugar solution. A significant increase in carbon monoxide production followed the infusion of urea. This result suggests that the treatment caused an increase in heme turnover. As taken from Bensinger TA et al. N. Engl. J. Med.; VOL 285 ISS Oct 28 1971, P995-997. Available at http://www.nejm.org/doi/full/10.1056/NEJM197110282851804

Tissue toxicity of intravenous solutions. A phlebographic and experimental study (Abstract).
A phlebographic study in 32 children has shown that i.v. infusion of invertose, glucose, Vamine and Intralipid but not saline, damages the tissues as judged from changes in the vessel walls, oedema and disturbances in the venous circulation. The addition of Heparin to the solutions markedly reduced the frequency of these untoward reactions. The toxicity of invertose, glucose, Vamine and Intralipid was studied experimentally in a biologic tissue: the cheek pouch of the hamster. All solutions particularly Vamine caused some damage to the microcirculation. In this study the factor mainly responsible for thrombophlebitis was the toxicity of the solution infused. Other factors such as surgical trauma, site of entry, local infection, temperature of solutions etc. played only a minor role. As taken from Enger E et al. Acta Paediatr Scand. 1976, Mar; 65(2):248-52. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgicmd=retrieve&db=pubmed&list_uids=816173&dopt=AbstractPlus

“10% soln of invert sugar buffered to pH 6.8 was found to cause significantly lower incidence of thrombophlebitis than soln of same strength with pH between 3.5 and 5.4 following intravenous injection of 500 ml quantities in 76 patients”

As taken from HSDB, 2002.

6.3. Nervous system

No data available to us at this time.

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

No data available to us at this time.

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 Sugars (invert sugar) 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 Sugars (invert sugar). Table below provides tested level(s) and specific endpoint(s).

Endpoint	**Tested level (ppm)**	**Reference**
Smoke chemistry	1,392	Carmines, 2002 & Rustemeier et al., 2002
	20,000 (No CAS) 48,000	JTI KB Study Report(s)
	19,975	Roemer et al, 2014
	49,000	Gaworski et al., 2011 & Coggins et al., 2011a
In vitro genotoxicity	1,392	Carmines, 2002 & Röemer et al., 2002
	70,000	Baker et al., 2004c
	20,000	Renne et al., 2006
	20,000 (No CAS)	JTI KB Study Report(s)
	48,700	fGLH Study Report (2010)
	19,975	Roemer et al, 2014
	49,000	Gaworski et al., 2011 & Coggins et al., 2011a

	1,392	Carmines, 2002 & Röemer et al., 2002
In vitro cytotoxicity	70,000	Baker et al., 2004c
	48,700	fGLH Study Report (2010)
	19,975	Roemer et al, 2014
	49,000	Gaworski et al., 2011 & Coggins et al., 2011a
Inhalation study	1,392	Carmines, 2002 & Vanscheeuwijck et al., 2002
	70,000	Baker et al., 2004c
	20,000	Renne et al., 2006
	20,000 (No CAS)	JTI KB Study Report(s)
	19,975	Schramke et al, 2014
	49,000	Gaworski et al., 2011 & Coggins et al., 2011a
Skin painting	20,000 ("Brown invert syrup", no CAS)	Gaworski et al., 1999
	20,000 (No CAS)	JTI KB Study Report(s)
In vivogenotoxicity	19,975	Schramke et al, 2014

Toxicological evaluation of honey as an ingredient added to cigarette tobacco (Abstract). A tiered testing strategy has been developed to evaluate the potential for new ingredients, tobacco processes, and technological developments to increase or reduce the biological activity that results from burning tobacco. In the manufacture of cigarettes, honey is used as a casing ingredient to impart both aroma and taste. The primary objective of this document is to summarize and interpret chemical and toxicological studies that have been conducted to evaluate the potential impact of honey on the biological activity of either mainstream cigarette smoke or cigarette smoke condensate. As part of ongoing stewardship efforts, cigarettes produced with honey (5% wet weight) as an alternative to invert sugar in tobacco casing material were subjected to extensive evaluation. Principal components of this evaluation were a determination of selected mainstream smoke constituent yields, Ames assay, sister chromatid exchange assay in Chinese hamster ovary cells, a 30-wk dermal tumor promotion evaluation of cigarette smoke condensate in SENCAR mice, and a 13-wk inhalation study of cigarette smoke in Sprague-Dawley rats. Comparative analytical evaluations demonstrated that the substitution of honey for invert sugar as a casing material in cigarettes had no significant impact on mainstream smoke chemistry. In addition, in vitro and in vivo studies demonstrated that cigarettes containing tobacco cased with honey had comparable

biological activity to cigarettes containing invert sugar. Collectively, these data demonstrate that the use of honey as an alternative casing material in the manufacture of cigarettes does not alter the potential toxicity of cigarette smoke condensate (CSC) or cigarette smoke; therefore the use of honey as an ingredient added to cigarette tobacco is acceptable from a toxicological perspective (Stavanja MS et al., 2003).

Safety assessment of high fructose corn syrup (HFCS) as an ingredient added to cigarette tobacco (Abstract). A tiered testing strategy has been developed to evaluate the potential for new ingredients, tobacco processes, and technological developments to alter the biological activity that results from burning tobacco. A series of studies was initially conducted with cigarettes containing 3% high fructose corn syrup (HFCS) as an alternate tobacco casing material to corn syrup/invert sugar, including determination of selected mainstream cigarette smoke (MS) constituent yields, Ames assay, sister chromatid exchange (SCE) assay in Chinese hamster ovary (CHO) cells, a 30-week dermal tumor-promotion evaluation of cigarette smoke condensate (CSC) in SENCAR mice, and a 13-week subchronic inhalation study of MS in Sprague-Dawley rats. A second series of studies was conducted with cigarettes containing 3%, 4% and 5% HFCS including MS chemistry, Ames assay, SCE assay in CHO cells, and a neutral red cytotoxicity assays. Collectively, mainstream smoke chemistry, genotoxicity, dermal tumor-promotion, and inhalation toxicity studies demonstrated no differences between cigarettes with 3% HFCS and cigarettes with 3% corn syrup/invert sugar. Also, mainstream smoke chemistry and genotoxicity of cigarettes with 4% and 5% HFCS were not different from cigarettes with 3% HFCS. In conclusion, the addition of up to 5% HFCS to cigarette does not alter the mainstream smoke chemistry or biological activity of mainstream smoke or mainstream smoke condensate as compared to cigarettes with 3% corn syrup/invert sugar with regard to the parameters investigated and presented (Stavanja MS et al., 2006).

Sugars, such as sucrose or invert sugar, have been used as tobacco ingredients in American-blend cigarettes to replenish the sugars lost during curing of the Burley component of the blended tobacco in order to maintain a balanced flavor. Chemical-analytical studies of the mainstream smoke of research cigarettes with various sugar application levels revealed that most of the smoke constituents determined did not show any sugar-related changes in yields (per mg nicotine), while ten constituents were found to either increase (formaldehyde, acrolein, 2-butanone, isoprene, benzene, toluene, benzo[k]fluoranthene) or decrease (4-aminobiphenyl, N-nitrosodimethylamine, N-nitrosonornicotine) in a statistically significant manner with increasing sugar application levels. Such constituent yields were modelled into constituent uptake distributions using simulations of nicotine uptake distributions generated on the basis of published nicotine biomonitoring data, which were multiplied by the constituent/nicotine ratios determined in the current analysis. These simulations revealed extensive overlaps for the constituent uptake distributions with and without sugar application. Moreover, the differences in smoke composition did not lead to relevant changes in the activity in in vitro and in vivo assays. The potential impact of using sugars as tobacco ingredients was further assessed in an indirect manner by comparing published data from markets with predominantly American-blend or Virginiatype (no added sugars) cigarettes. No relevant difference was found between these markets for smoking prevalence, intensity, some markers of dependence, nicotine uptake, or mortality from smoking-related lung cancer and chronic obstructive pulmonary disease. In conclusion, thorough examination of the data available suggests that the use of sugars as ingredients in cigarette tobacco does not increase the inherent risk and harm of cigarette smoking (Roemer et al., 2012).

9. Heated/vapor emissions toxicity

No data available to us at this time.

10. Ecotoxicity

10.1. Environmental fate

No data available to us at this time.

10.2. Aquatic toxicity

No data available to us at this time.

10.3. Sediment toxicity

No data available to us at this time.

10.4. Terrestrial toxicity

Honey bee foraging preferences, effects of sugars, and fruit fly toxic bait components (Abstract).
Field tests were carried out to evaluate the repellency of the Dow AgroSciences fruit fly toxic bait GF-120 (NF Naturalyte) to domestic honey bees (Apis mellifera L.). GF-120 is an organically registered attractive bait for tephritid fruit flies composed of spinosad, hydrolyzed protein (Solulys), high-fructose corn syrup (ADM CornSweet 42 high-fructose corn syrup, referred to as invertose sugar or invertose here), vegetable oils, adjuvants, humectants, and attractants. Tests were carried out with non-Africanized honey bees in February and March 2005 and 2007 during periods of maximum hunger for these bees. In all tests, bees were first trained to forage from plates of 30% honey-water (2005) or 30% invertose (2007). In 2005 bees were offered choices between honey-water and various bait components, including the complete toxic bait. In 2007, similar tests were performed except bees were attracted with 30% invertose then offered the bait components or complete bait as no-choice tests. Initially, the 2005 tests used all the components of GF-120 except the spinosad as the test bait. After we were convinced that bees would not collect or be contaminated by the bait, we tested the complete GF-120. Behavior of the bees indicated that during initial attraction and after switching the baits, the bait components and the complete bait were repellent to honey bees, but the honey-water remained attractive. Invertose was shown to be less attractive to bees, addition of Solulys eliminated almost all bee activity, and addition of ammonium acetate completely eliminated feeding in both choice and no-choice tests. These results confirm previous tests showing that bees do not feed on GF-120 and also show that honey bees are repelled by the fruit fly attractant components of the bait in field tests. As taken from Mangan RL and Moreno AT (2009). J Econ Entomol. Aug; 102(4):1472-81. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgicmd=retrieve&db=pubmed&list_uids=19736759&dopt=AbstractPlus

10.5. All other relevant types of ecotoxicity

No data available to us at this time.

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12. Other information

No data available to us at this time.

13. Last audited

May 2022

Inhalation Toxicology, 18:685–706, 2006
Copyright © Taylor and Francis Group, LLC
ISSN: 0895-8378 print / 1091-7691 online
DOI: 10.1080/08958370600742680

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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.

Received 2 January 2006; accepted 31 March 2006.
The authors are grateful to the following staff for their valuable contributions to this work: J. C. Blessing, M. L. Clark, K. M. Gideon, B. K. Hayden, J. D. Penner, J. T. Pierce, B. L. Thomas, and R. L. Thomas.
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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

(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)
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.

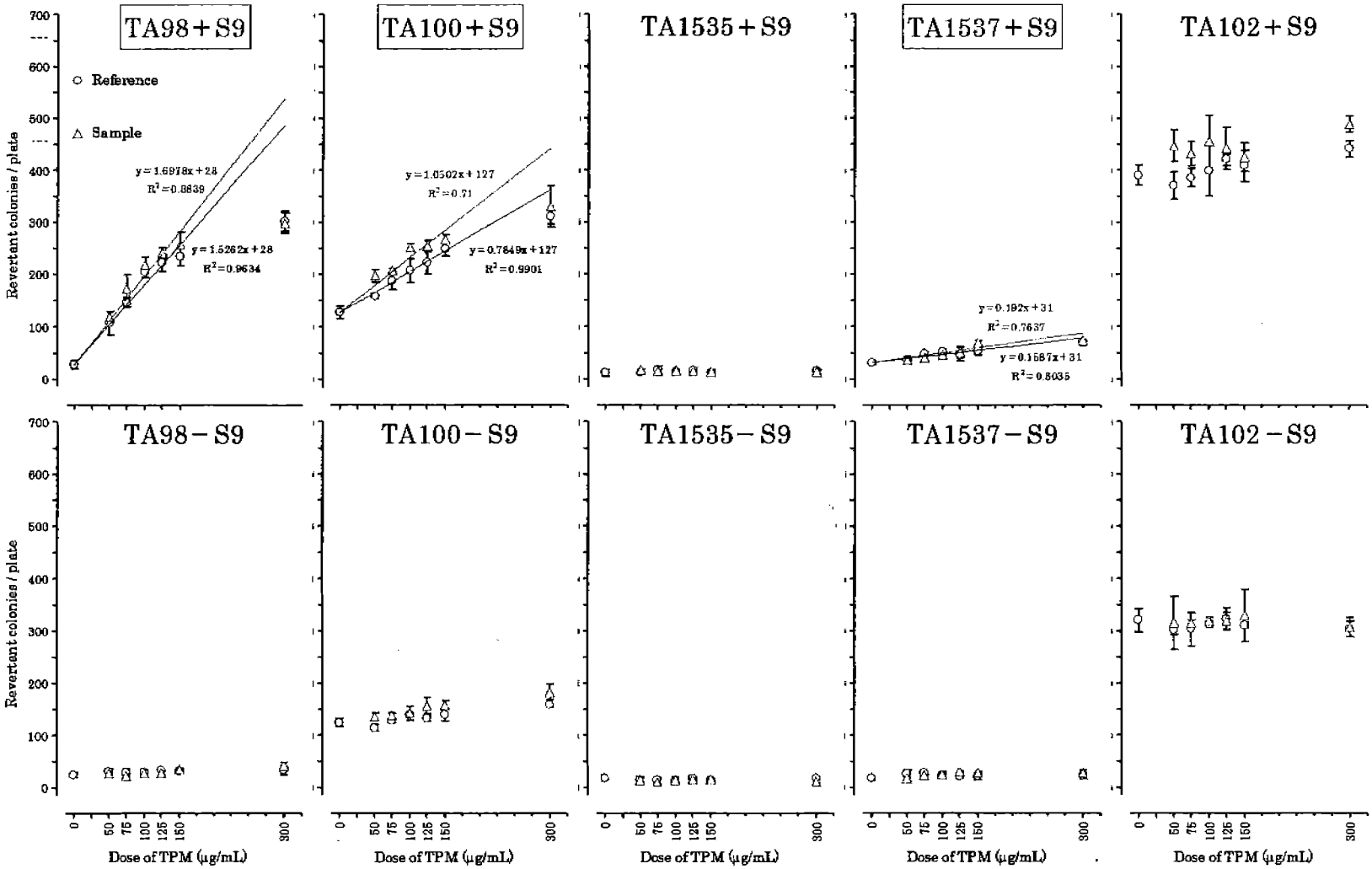
Body Weights and Clinical Observations

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.

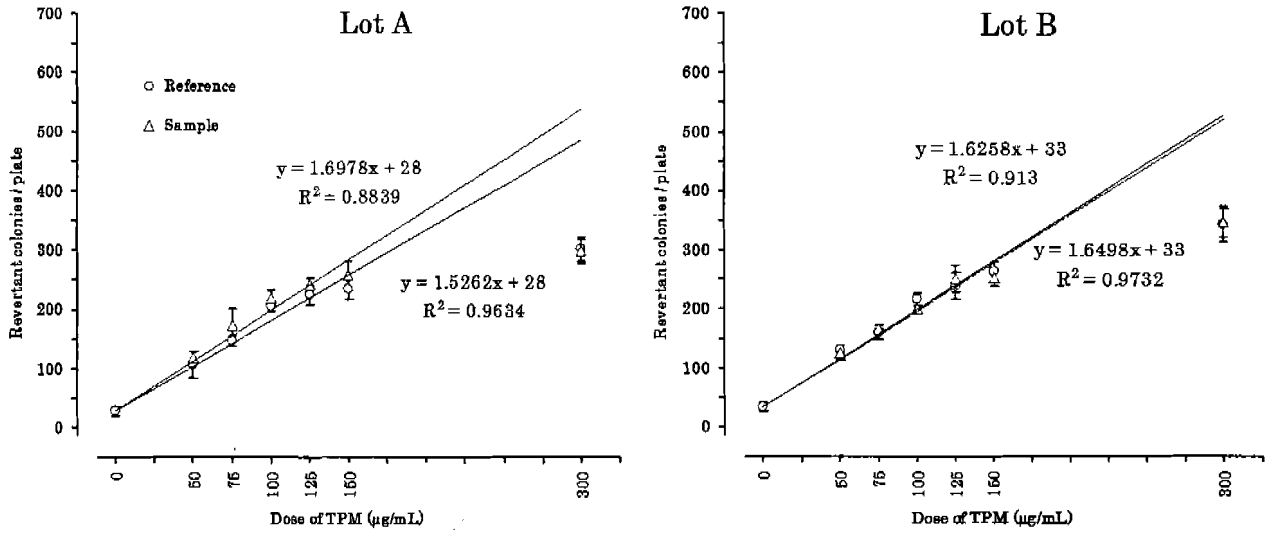
Organ Weights

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

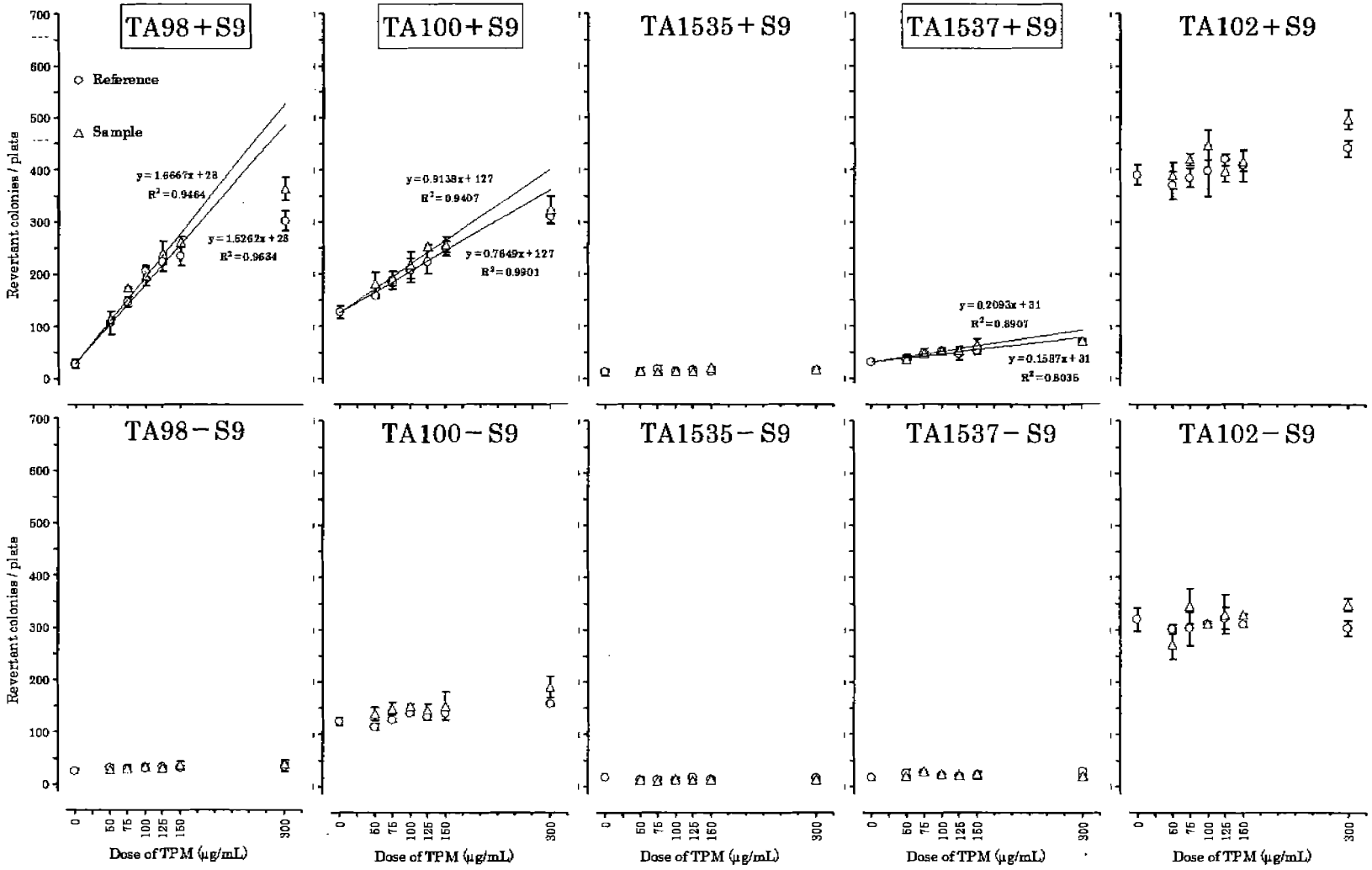
There were occasional statistically significant differences in hematology and clinical chemistry parameters from control values in groups exposed to smoke from test or reference cigarettes in both study 1 and study 2. These differences did not occur in a dose-response pattern and were well within ±2 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



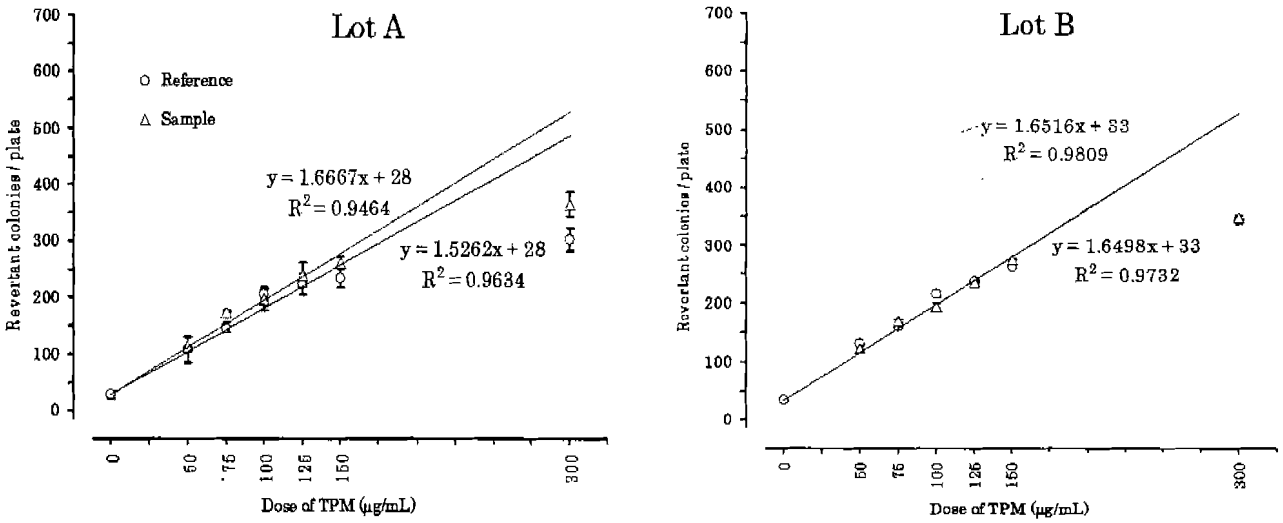
N=2. Only the first lot (Lot A) is indicated in this figure.
The second lot (Lot B) showed the same tendency as the first lot .

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

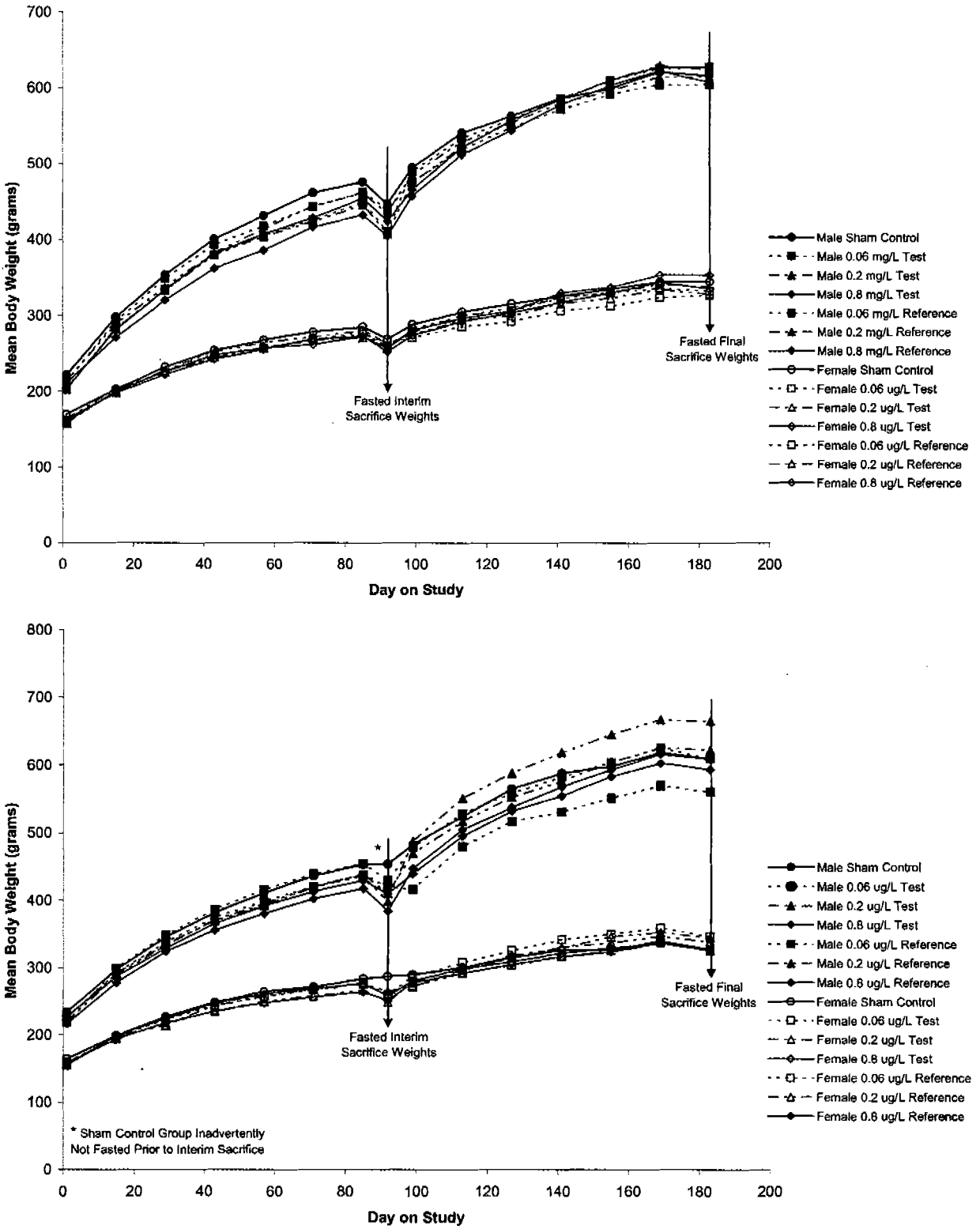


FIG. 5. Body weights, study 1 (top) and study 2 (bottom).

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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9 October 2017
EMA/CHMP/302620/2017 corr. 1*

Annex to the European Commission guideline on 'Excipients in the labelling and package leaflet of medicinal products for human use' (SANTE-2017-11668)

Excipients and information for the package leaflet

Agreed by CHMP Excipients Drafting Group	6 July 2017
Adopted by EMA Committee for Medicinal Products for Human Use (CHMP)	20 July 2017
Endorsed by European Commission's Notice to Applicants Group	4 October 2017
Date of publication	9 October 2017

This document updates and replaces the Annex previously included in the Guideline CPMP/463/00 Rev. 1.

It is an integral part of the European Commission guideline on 'Excipients in the labelling and package leaflet of medicinal products for human use' (SANTE-2017-11668).

Keywords	***Excipient, Package Leaflet, Labelling***

* See pages 23-25 for details.

Excipients and information for the package leaflet

Name	Updated on	Route of Administration	Threshold	Information for the Package Leaflet	Comments
Aprotinin		Topical	Zero	May cause hypersensitivity or severe allergic reactions.	The topical route in this case refers to sites that may have access to the circulation (e.g. wounds, body cavities etc.).
Arachis oil (peanut oil)		All	Zero	<Medicinal product> contains arachis oil (peanut oil). If you are allergic to peanut or soya, do not use this medicinal product.	Purified arachis oil may contain peanut protein. The PhEur monograph does not contain a test for residual protein. SmPC: contraindication.
Aspartame (E 951)	**09/10/2017**	Oral	Zero	This medicine contains x mg aspartame in each <dosage unit><unit volume> <which is equivalent to x mg/<weight><volume>>. Aspartame is a source of phenylalanine. It may be harmful if you have phenylketonuria (PKU), a rare genetic disorder in which phenylalanine builds up because the body cannot remove it properly.	Aspartame is hydrolysed in the gastrointestinal tract when orally ingested. One of the major hydrolysis products is phenylalanine. Information to consider for the SmPC: Neither non-clinical nor clinical data are available to assess aspartame use in infants below 12 weeks of age.
Azo colouring agents e.g.: Tartrazine (E 102) Sunset yellow FCF (E 110) Azorubine, carmoisine (E 122) Amaranth (E 123) Ponceau 4R, cochineal Red A (E 124) Brilliant black BN, black PN (E 151)		Oral	Zero	May cause allergic reactions.	
Balsam of Peru		Topical	Zero	May cause skin reactions.	
Benzalkonium chloride	**09/10/2017**	All	Zero	This medicine contains x mg benzalkonium chloride in each <dosage unit><unit volume> <which is equivalent to x mg/<weight><volume>>.	

Name	Updated on	Route of Administration	Threshold	Information for the Package Leaflet	Comments
Benzalkonium chloride	09/10/2017	Ocular	Zero	Benzalkonium chloride may be absorbed by soft contact lenses and may change the colour of the contact lenses. You should remove contact lenses before using this medicine and put them back 15 minutes afterwards. Benzalkonium chloride may also cause eye irritation, especially if you have dry eyes or disorders of the cornea (the clear layer at the front of the eye). If you feel abnormal eye sensation, stinging or pain in the eye after using this medicine, talk to your doctor.	From the limited data available, there is no difference in the adverse event profile in children compared to adults. Generally, however, eyes in children show a stronger reaction for a given stimulus than the adult eye. Irritation may have an effect on treatment adherence in children. Benzalkonium chloride has been reported to cause eye irritation, symptoms of dry eyes and may affect the tear film and corneal surface. Should be used with caution in dry eye patients and in patients where the cornea may be compromised. Patients should be monitored in case of prolonged use.
Benzalkonium chloride	09/10/2017	Nasal	Zero	Benzalkonium chloride may cause irritation or swelling inside the nose, especially if used for a long time.	Long-term use may cause oedema of the nasal mucosa.
Benzalkonium chloride	09/10/2017	Inhalation	Zero	Benzalkonium chloride may cause wheezing and breathing difficulties (bronchospasm), especially if you have asthma.	
Benzalkonium chloride	09/10/2017	Cutaneous	Zero	Benzalkonium chloride may irritate the skin. You should not apply this medicine to the breasts if you are breast-feeding because the baby may take it in with your milk.	Use during pregnancy and lactation is not expected to be associated with harmful effects to the mother as cutaneous absorption of benzalkonium chloride is minimal. Not for application to mucosa.
Benzalkonium chloride	09/10/2017	Oromucosal, rectal and vaginal	Zero	Benzalkonium chloride may cause local irritation.	
Benzoic acid (E 210) and benzoates e.g.: Sodium benzoate (E 211) Potassium benzoate (E 212)	09/10/2017	All	Zero	This medicine contains x mg <benzoic acid/benzoate salt> in each <dosage unit><unit volume> <which is equivalent to x mg/<weight><volume>>.	

Name	Updated on	Route of Administration	Threshold	Information for the Package Leaflet	Comments
Benzoic acid (E 210) and benzoates e.g.: Sodium benzoate (E 211) Potassium benzoate (E 212)	09/10/2017	Oral, parenteral	Zero	<Benzoic acid/Benzoate salt> may increase jaundice (yellowing of the skin and eyes) in newborn babies (up to 4 weeks old).	Increase in bilirubinaemia following its displacement from albumin may increase neonatal jaundice which may develop into kernicterus (non-conjugated bilirubin deposits in the brain tissue).
Benzoic acid (E 210) and benzoates e.g.: Sodium benzoate (E 211) Potassium benzoate (E 212)	09/10/2017	Topical	Zero	<Benzoic acid/Benzoate salt> may cause local irritation.	May cause non-immunologic immediate contact reactions by a possible cholinergic mechanism.
Benzoic acid (E 210) and benzoates e.g.: Sodium benzoate (E 211) Potassium benzoate (E 212)	09/10/2017	Topical	Zero	<Benzoic acid/Benzoate salt> may increase jaundice (yellowing of the skin and eyes) in newborn babies (up to 4 weeks old).	Absorption through the immature skin of neonates is significant.
Benzyl alcohol	09/10/2017	All	Zero	This medicine contains x mg benzyl alcohol in each <dosage unit><unit volume> <which is equivalent to x mg/<weight><volume>>. Benzyl alcohol may cause allergic reactions.	
Benzyl alcohol	09/10/2017	Oral, parenteral	Zero	Benzyl alcohol has been linked with the risk of severe side effects including breathing problems (called “gasping syndrome”) in young children. Do not give to your newborn baby (up to 4 weeks old), unless recommended by your doctor.	Intravenous administration of benzyl alcohol has been associated with serious adverse events and death in neonates (“gasping syndrome”). The minimum amount of benzyl alcohol at which toxicity may occur is not known. Warning in section 4.4 in the SmPC should be given if used in neonates.
Benzyl alcohol	09/10/2017	Oral, parenteral	Zero	Do not use for more than a week in young children (less than 3 years old), unless advised by your doctor or pharmacist.	Increased risk due to accumulation in young children.
Benzyl alcohol	09/10/2017	Oral, parenteral	Zero	Ask your doctor or pharmacist for advice if you are pregnant or breast-feeding. This is because large amounts of benzyl alcohol can build-up in your body and may cause side effects (called “metabolic acidosis”).	

Name	Updated on	Route of Administration	Threshold	Information for the Package Leaflet	Comments
Benzyl alcohol	**09/10/2017**	Oral, parenteral	Zero	Ask your doctor or pharmacist for advice if you have a liver or kidney disease. This is because large amounts of benzyl alcohol can build-up in your body and may cause side effects (called “metabolic acidosis”).	High volumes should be used with caution and only if necessary, especially in subjects with liver or kidney impairment because of the risk of accumulation and toxicity (metabolic acidosis).
Benzyl alcohol	**09/10/2017**	Topical	Zero	Benzyl alcohol may cause mild local irritation.	
Bergamot oil (containing bergapten)		Topical	Zero	May increase sensitivity to UV light (natural and artificial sunlight).	Does not apply when bergapten is shown to be absent from the oil.
Boric acid (and borates)	**09/10/2017**	All	1 mg B/day*	Do not give to a child less than 2 years old as this medicine contains boron and may impair fertility in the future.	* 1 mg B (Boron) = 5.7 mg boric acid. See Q&A document (EMA/CHMP/619104/2013) for further calculations. Amount of boron per age group which may impair fertility if exceeded: **Age** **Safety limit** < 2 years 1 mg B/day < 12 years 3 mg B/day < 18 years** 7 mg B/day ≥ 18 years** 10 mg B/day ** This amount may also cause harm to the unborn child.

Name	Updated on	Route of Administration	Threshold	Information for the Package Leaflet	Comments
Boric acid (and borates)	**09/10/2017**	All	3 mg B/day*	Do not give to a child less than 12 years old as this medicine contains boron and may impair fertility in the future.	* 1 mg B (Boron) = 5.7 mg boric acid. See Q&A document (EMA/CHMP/619104/2013) for further calculations. Amount of boron per age group which may impair fertility if exceeded: **Age** **Safety limit** < 2 years 1 mg B/day < 12 years 3 mg B/day < 18 years** 7 mg B/day ≥ 18 years** 10 mg B/day ** This amount may also cause harm to the unborn child.
Boric acid (and borates)	**09/10/2017**	All	7 mg B/day*	Do not give to a child less than 18 years old as this medicine contains boron and may impair fertility in the future. If you are pregnant, talk to your doctor before taking this medicine as it contains boron which may be harmful to your baby.	* 1 mg B (Boron) = 5.7 mg boric acid. See Q&A document (EMA/CHMP/619104/2013) for further calculations. Amount of boron per age group which may impair fertility if exceeded: **Age** **Safety limit** < 2 years 1 mg B/day < 12 years 3 mg B/day < 18 years** 7 mg B/day ≥ 18 years** 10 mg B/day ** This amount may also cause harm to the unborn child.
Bronopol		Topical	Zero	May cause local skin reactions (e.g. contact dermatitis).	
Butylated hydroxyanisole (E 320)		Topical	Zero	May cause local skin reactions (e.g. contact dermatitis), or irritation to the eyes and mucous membranes.	

Name	Updated on	Route of Administration	Threshold	Information for the Package Leaflet	Comments
Butylated hydroxytoluene (E 321)		Topical	Zero	May cause local skin reactions (e.g. contact dermatitis), or irritation to the eyes and mucous membranes.	
Cetostearyl alcohol including Cetyl alcohol		Topical	Zero	May cause local skin reactions (e.g. contact dermatitis).	
Chlorocresol		Topical, parenteral	Zero	May cause allergic reactions.	
Cyclodextrins e.g.: Alfadex Betadex (E 459) γ-cyclodextrin Sulfobutyl-ether-β-cyclodextrin (SBE-β-CD) Hydroxypropyl betadex Randomly methylated β-cyclodextrin (RM-β-CD)	**09/10/2017**	All	20 mg/kg/day	This medicine contains x mg cyclodextrin(s) in each <dosage unit><unit volume> <which is equivalent to x mg/<weight><volume>>. Do not use in children less than 2 years old unless recommended by your doctor.	Cyclodextrins (CDs) are excipients which can influence the properties (such as toxicity or skin penetration) of the active substance and other medicines. Safety aspects of CDs have been considered during the development and safety assessment of the drug product, and are clearly stated in the SmPC. There is insufficient information on the effects of CDs in children < 2 years old. Therefore, a case by case judgement should be made regarding the risk/benefit for the patient. Based on animal studies and human experience, harmful effects of CDs are not to be expected at doses below 20 mg/kg/day.
Cyclodextrins e.g.: Alfadex Betadex (E 459) γ-cyclodextrin Sulfobutyl-ether-β-cyclodextrin (SBE-β-CD) Hydroxypropyl betadex Randomly methylated β-cyclodextrin (RM-β-CD)	**09/10/2017**	Oral	200 mg/kg/day	Cyclodextrins may cause digestive problems such as diarrhoea.	At high doses cyclodextrins can cause reversible diarrhoea and cecal enlargement in animals.

Name	Updated on	Route of Administration	Threshold	Information for the Package Leaflet	Comments
Cyclodextrins e.g.: Alfadex Betadex (E 459) γ-cyclodextrin Sulfobutyl-ether-β-cyclodextrin (SBE-β-CD) Hydroxypropyl betadex Randomly methylated β-cyclodextrin (RM-β-CD)	09/10/2017	Parenteral	200 mg/kg/day and use for > 2 weeks	If you have a kidney disease, talk to your doctor before you receive this medicine.	In children less than 2 years, the lower glomerular function may protect against renal toxicity, but can lead to higher blood levels of cyclodextrins. In patients with moderate to severe renal dysfunction accumulation of cyclodextrins may occur.
Dimethyl sulphoxide		Topical	Zero	May be irritant to the skin.	
Ethanol		Oral, parenteral	Less than 100 mg per dose	This medicinal product contains small amounts of ethanol (alcohol), less than 100 mg per <dose>.	This statement is to provide reassurance to parents and children concerning the low levels of alcohol in the product.
Ethanol		Oral, parenteral	100 mg per dose	This medicinal product contains ... vol % ethanol (alcohol), i.e. up to ... mg per <dose>, equivalent to ... ml beer, ... ml wine per <dose>. Harmful for those suffering from alcoholism. To be taken into account in pregnant or breast-feeding women, children and high-risk groups such as patients with liver disease, or epilepsy.	The package leaflet should give the equivalent volume of beer and wine, nominally calculated assuming 5% vol and 12% vol ethanol respectively. Separate warning statements may be needed in different parts of the PL.
Ethanol		Oral, parenteral	3 g per dose	This medicinal product contains ... vol % ethanol (alcohol), i.e. up to ... mg per <dose>, equivalent to ... ml beer, ... ml wine per <dose>. Harmful for those suffering from alcoholism. To be taken into account in pregnant or breast-feeding women, children and high-risk groups such as patients with liver disease or epilepsy. The amount of alcohol in this medicinal product may alter the effects of other medicines. The amount of alcohol in this medicinal product may impair your ability to drive or use machines.	

Name	Updated on	Route of Administration	Threshold	Information for the Package Leaflet	Comments
Formaldehyde		Topical	Zero	May cause local skin reactions (e.g. contact dermatitis).	
Formaldehyde		Oral	Zero	May cause stomach upset and diarrhea.	
Fragrances containing allergens* **(See appendix)**	**09/10/2017**	Topical	Zero	This medicine contains fragrance with <allergen(s)>*. <Allergen(s)>* may cause allergic reactions.	*< >: fragrance allergens listed in appendix. In addition to allergic reactions in sensitised patients, non-sensitised patients may become sensitised. Benzyl alcohol is listed as one of the 26 fragrance allergens but can also be used as an excipient. When benzyl alcohol is used as an excipient (in addition to a fragrance or not), the label of this excipient applies.
Fructose	**09/10/2017**	Oral, parenteral	Zero	This medicine contains x mg fructose in each <dosage unit><unit volume> <which is equivalent to x mg/<weight><volume>>.	The additive effect of concomitantly administered products containing fructose (or sorbitol) and dietary intake of fructose (or sorbitol) should be taken into account.
Fructose	**09/10/2017**	Oral	Zero	*[If the medicine is in contact with teeth (e.g. oral liquids, lozenges or chewable tablets) and is intended for long term use:]* Fructose may damage teeth.	Oral products used frequently or over a long period of time, e.g. for two weeks or longer.
Fructose	**09/10/2017**	Intravenous (IV)	Zero	If you (or your child) have hereditary fructose intolerance (HFI), a rare genetic disorder, you (or your child) must not receive this medicine. Patients with HFI cannot break down fructose in this medicine, which may cause serious side effects. You must tell your doctor before receiving this medicine if you (or your child) have HFI or if your child can no longer take sweet foods or drinks because they feel sick, vomit or get unpleasant effects such as bloating, stomach cramps or diarrhoea.	Patients with hereditary fructose intolerance (HFI) must not be given this medicine unless strictly necessary. Babies and young children (below 2 years of age) may not yet be diagnosed with hereditary fructose intolerance (HFI). Medicines (containing fructose) given intravenously may be life-threatening and must be contraindicated in this population unless there is an overwhelming clinical need and no alternatives are available. A detailed history with regard to HFI symptoms has to be taken of each patient prior to being given this medicinal product.

Name	Updated on	Route of Administration	Threshold	Information for the Package Leaflet	Comments
Fructose	09/10/2017	Oral, parenteral (other than IV)	5 mg/kg/day	If your doctor has told you that you (or your child) have an intolerance to some sugars or if you have been diagnosed with hereditary fructose intolerance (HFI), a rare genetic disorder in which a person cannot break down fructose, talk to your doctor before you (or your child) take or receive this medicine.	Patients with hereditary fructose intolerance (HFI) should not take/be given this medicinal product.
Galactose		Oral, parenteral	Zero	If you have been told by your doctor that you have an intolerance to some sugars, contact your doctor before taking this medicinal product.	SmPC proposal: Patients with rare hereditary problems of galactose intolerance e.g. galactosaemia<, or glucose-galactose malabsorption> should not take this medicine.
Galactose		Oral, parenteral	5 g	Contains x g galactose per dose. This should be taken into account in patients with diabetes mellitus.	
Glucose		Oral	Zero	If you have been told by your doctor that you have an intolerance to some sugars, contact your doctor before taking this medicinal product.	SmPC proposal: Patients with rare glucose-galactose malabsorption should not take this medicine.
Glucose		Oral, parenteral	5 g	Contains x g glucose per dose. This should be taken into account in patients with diabetes mellitus.	
Glucose		Oral liquids, lozenges and chewable tablets	Zero	May be harmful to the teeth.	Information to be included only when the medicinal product may be intended for chronic use, e.g. for two weeks or more.
Glycerol (E 422)		Oral	10 g per dose	May cause headache, stomach upset and diarrhea.	
Glycerol (E 422)		Rectal	1 g	May have a mild laxative effect.	
Heparin (as an excipient)		Parenteral	Zero	May cause allergic reactions and reduced blood cell counts which may affect the blood clotting system. Patients with a history of heparin-induced allergic reactions should avoid the use of heparin-containing medicines.	
Invert sugar		Oral	Zero	If you have been told by your doctor that you have an intolerance to some sugars, contact your doctor before taking this medicinal product.	SmPC proposal: Patients with rare hereditary problems of fructose intolerance or glucose-galactose malabsorption should not take this medicine.

Name	Updated on	Route of Administration	Threshold	Information for the Package Leaflet	Comments
Invert sugar		Oral	5 g	Contains x g of a mixture of fructose and glucose per dose. This should be taken into account in patients with diabetes mellitus.	
Invert sugar		Oral liquids, lozenges and chewable tablets	Zero	May be harmful to the teeth.	Information to be included only when the medicinal product may be intended for chronic use, e.g. for two weeks or more.
Lactitol (E 966)		Oral	Zero	If you have been told by your doctor that you have an intolerance to some sugars contact your doctor before taking this medicinal product.	SmPC proposal: Patients with rare hereditary problems of fructose intolerance, galactose intolerance, galactosaemia or glucose-galactose malabsorption should not take this medicine.
Lactitol (E 966)		Oral	10 g	May have a mild laxative effect. Calorific value 2.1 kcal/g lactitol.	
Lactose		Oral	Zero	If you have been told by your doctor that you have an intolerance to some sugars, contact your doctor before taking this medicinal product.	SmPC proposal: Patients with rare hereditary problems of galactose intolerance, total lactase deficiency or glucose-galactose malabsorption should not take this medicine.
Lactose		Oral	5 g	Contains x g lactose (x/2 g glucose and x/2 g galactose) per dose. This should be taken into account in patients with diabetes mellitus.	
Latex **Natural Rubber (latex)**		All	Zero	The container of this medicinal product contains latex rubber. May cause severe allergic reactions.	Not a typical excipient, but a warning is considered necessary.
Macrogolglycerol ricinoleate (castor oil polyoxyl) **Macrogolglycerol hydroxystearate** (castor oil polyoxyl hydrogenated)		Parenteral	Zero	May cause severe allergic reactions.	
Macrogolglycerol ricinoleate (castor oil polyoxyl) **Macrogolglycerol hydroxystearate** (castor oil polyoxyl hydrogenated)		Oral	Zero	May cause stomach upset and diarrhea.	

Name	Updated on	Route of Administration	Threshold	Information for the Package Leaflet	Comments
Macrogolglycerol ricinoleate (castor oil polyoxyl) **Macrogolglycerol hydroxystearate** (castor oil polyoxyl hydrogenated)		Topical	Zero	May cause skin reactions.	
Maltitol (E 965) **Isomalt (E 953)** (isomaltitol) **Maltitol liquid** (hydrogenated glucose syrup)		Oral	Zero	If you have been told by your doctor that you have an intolerance to some sugars, contact your doctor before taking this medicinal product.	SmPC proposal: Patients with rare hereditary problems of fructose intolerance should not take this medicine.
Maltitol (E 965) **Isomalt (E 953)** (isomaltitol) **Maltitol liquid** (hydrogenated glucose syrup)		Oral	10 g	May have a mild laxative effect. Calorific value 2.3 kcal/g <maltitol><isomalt>.	
Mannitol (E 421)		Oral	10 g	May have a mild laxative effect.	
Organic mercury compounds e.g.: Thiomersal Phenylmercuric nitrate/acetate/borate		Ocular	Zero	May cause allergic reactions.	See EMEA Public Statement, 8 July 1999, Ref. EMEA/20962/99
Organic mercury compounds e.g.: Thiomersal Phenylmercuric nitrate/acetate/borate		Topical	Zero	May cause local skin reactions (e.g. contact dermatitis) and discolouration.	

Name	Updated on	Route of Administration	Threshold	Information for the Package Leaflet	Comments
Organic mercury compounds e.g.: Thiomersal Phenylmercuric nitrate/acetate/borate		Parenteral	Zero	This medicinal product contains (thiomersal) as a preservative and it is possible that <you/your child> may experience an allergic reaction. Tell your doctor if <you/your child> have/has any known allergies.	See EMEA Public Statement, 8 July 1999, Ref. EMEA/20962/99
Organic mercury compounds e.g.: Thiomersal Phenylmercuric nitrate/acetate/borate		Parenteral	Zero	Tell your doctor if you/your child have/has experienced any health problems after previous administration of a vaccine.	Additional statement to be mentioned for vaccines.
Parahydroxybenzoates and their esters e.g.: Ethyl p-hydroxybenzoate (E 214) Sodium ethyl p-hydroxybenzoate (E 215) Propyl p-hydroxybenzoate Sodium propyl p-hydroxybenzoate Methyl p-hydroxybenzoate (E 218) Sodium methyl p-hydroxybenzoate (E 219)		Oral Ocular Topical	Zero	May cause allergic reactions (possibly delayed).	

Name	Updated on	Route of Administration	Threshold	Information for the Package Leaflet	Comments
Parahydroxybenzoates and their esters e.g.: Ethyl p-hydroxybenzoate (E 214) Sodium ethyl p-hydroxybenzoate (E 215) Propyl p-hydroxybenzoate Sodium propyl p-hydroxybenzoate Methyl p-hydroxybenzoate (E 218) Sodium methyl p-hydroxybenzoate (E 219)		Parenteral Inhalation	Zero	May cause allergic reactions (possibly delayed), and exceptionally, bronchospasm.	
Phenylalanine	**09/10/2017** *Corrigendum 19/11/2018*	All	Zero	This medicine contains x mg phenylalanine in each <dosage unit><unit volume> <which is equivalent to x mg/<weight><volume>>. Phenylalanine may be harmful if you have phenylketonuria (PKU), a rare genetic disorder in which phenylalanine builds up because the body cannot remove it properly.	
Phosphate buffers	**09/10/2017**	Ocular	Zero	This medicine contains x mg phosphates in each <dosage unit><unit volume> <which is equivalent to x mg/<weight><volume>>. If you suffer from severe damage to the clear layer at the front of the eye (the cornea), phosphates may cause in very rare cases cloudy patches on the cornea due to calcium build-up during treatment.	Corresponding SmPC statement in Section 4.8 (Undesirable effects): “Cases of corneal calcification have been reported very rarely in association with the use of phosphate containing eye drops in some patients with significantly damaged corneas.”
Potassium		Parenteral	Less than 1 mmol per dose	This medicine contains potassium, less than 1 mmol (39 mg) per <dose>, i.e. essentially ‘potassium-free’.	Information relates to a threshold based on the total amount of K^+ in the medicinal product. It is especially relevant to products used in paediatric doses, to provide information to prescribers and reassurance to parents concerning the low level of K^+ in the product.

Name	Updated on	Route of Administration	Threshold	Information for the Package Leaflet	Comments
Potassium		Oral, parenteral	1 mmol per dose	This medicine contains x mmol (or y mg) potassium per <dose>. To be taken into consideration by patients with reduced kidney function or patients on a controlled potassium diet.	
Potassium		Intravenous (IV)	30 mmol/l	May cause pain at the site of injection.	
Propylene glycol (E 1520) and esters of propylene glycol	**09/10/2017**	All	1 mg/kg/day	This medicine contains x mg propylene glycol in each <dosage unit><unit volume> <which is equivalent to x mg/<weight><volume>>.	
Propylene glycol (E 1520) and esters of propylene glycol	**09/10/2017**	Oral, parenteral	1 mg/kg/day	If your baby is less than 4 weeks old, talk to your doctor or pharmacist before giving them this medicine, in particular if the baby is given other medicines that contain propylene glycol or alcohol.	Co-administration with any substrate for alcohol dehydrogenase such as ethanol may induce serious adverse effects in neonates.
Propylene glycol (E 1520) and esters of propylene glycol	**09/10/2017**	Oral, parenteral	50 mg/kg/day	If your child is less than 5 years old, talk to your doctor or pharmacist before giving them this medicine, in particular if they use other medicines that contain propylene glycol or alcohol.	Co-administration with any substrate for alcohol dehydrogenase such as ethanol may induce adverse effects in children less than 5 years old.
Propylene glycol (E 1520) and esters of propylene glycol	**09/10/2017**	Oral, parenteral	50 mg/kg/day	If you are pregnant or breast-feeding, do not take this medicine unless recommended by your doctor. Your doctor may carry out extra checks while you are taking this medicine.	While propylene glycol has not been shown to cause reproductive or developmental toxicity in animals or humans, it may reach the foetus and was found in milk. As a consequence, administration of propylene glycol to pregnant or lactating patients should be considered on a case by case basis.
Propylene glycol (E 1520) and esters of propylene glycol	**09/10/2017**	Oral, parenteral	50 mg/kg/day	If you suffer from a liver or kidney disease, do not take this medicine unless recommended by your doctor. Your doctor may carry out extra checks while you are taking this medicine.	Medical monitoring is required in patients with impaired renal or hepatic functions because various adverse events attributed to propylene glycol have been reported such as renal dysfunction (acute tubular necrosis), acute renal failure and liver dysfunction.

Name	Updated on	Route of Administration	Threshold	Information for the Package Leaflet	Comments
Propylene glycol (E 1520) and esters of propylene glycol	**09/10/2017**	Oral, parenteral	500 mg/kg/day	Propylene glycol in this medicine can have the same effects as drinking alcohol and increase the likelihood of side effects. Do not use this medicine in children less than 5 years old. Use this medicine only if recommended by a doctor. Your doctor may carry out extra checks while you are taking this medicine.	Various adverse events, such as hyperosmolality, lactic acidosis; renal dysfunction (acute tubular necrosis), acute renal failure; cardiotoxicity (arrhythmia, hypotension); central nervous system disorders (depression, coma, seizures); respiratory depression, dyspnoea; liver dysfunction; haemolytic reaction (intravascular haemolysis) and haemoglobinuria; or multisystem organ dysfunction, have been reported with high doses or prolonged use of propylene glycol. Therefore doses higher than 500 mg/kg/day may be administered in children > 5 years old but will have to be considered case by case. Adverse events usually reverse following weaning off of propylene glycol, and in more severe cases following hemodialysis. Medical monitoring is required.
Propylene glycol (E 1520) and esters of propylene glycol	**09/10/2017**	Cutaneous	50 mg/kg/day	Propylene glycol may cause skin irritation. Do not use this medicine in babies less than 4 weeks old with open wounds or large areas of broken or damaged skin (such as burns) without talking to your doctor or pharmacist.	
Propylene glycol (E 1520) and esters of propylene glycol	**09/10/2017**	Cutaneous	500 mg/kg/day	Propylene glycol may cause skin irritation. Because this medicine contains propylene glycol, do not use it on open wounds or large areas of broken or damaged skin (such as burns) without checking with your doctor or pharmacist.	
Sesame oil		All	Zero	May rarely cause severe allergic reactions.	

Name	Updated on	Route of Administration	Threshold	Information for the Package Leaflet	Comments
Sodium	**09/10/2017**	Oral, parenteral	Less than 1 mmol (23 mg) per dose	This medicine contains less than 1 mmol sodium (23 mg) per <dosage unit><unit volume>, that is to say essentially 'sodium-free'.	1 mmol of sodium (Na) = 23 mg Na = 58.4 mg salt (NaCl). Information relates to a threshold based on the total amount of sodium in the medicinal product. It is especially relevant to products used in children or in patients on a low sodium diet, to provide information to prescribers and reassurance to parents or patients concerning the low level of sodium in the product.
Sodium	**09/10/2017**	Oral, parenteral	1 mmol (23 mg) per dose	This medicine contains x mg sodium (main component of cooking/table salt) in each <dosage unit><unit volume>. This is equivalent to y% of the recommended maximum daily dietary intake of sodium for an adult.	For parenterals with variable (e.g. weight-based) dosing sodium content may be expressed in mg per vial. Proposed wording for SmPC: "This medicinal product contains x mg sodium per <dosage unit>, equivalent to y% of the WHO recommended maximum daily intake of 2 g sodium for an adult."

Name	Updated on	Route of Administration	Threshold	Information for the Package Leaflet	Comments
Sodium	**09/10/2017**	Oral, parenteral	17 mmol (391 mg) in the maximum daily dose	Talk to your doctor or pharmacist if you need <Z> or more <dosage units> daily for a prolonged period, especially if you have been advised to follow a low salt (sodium) diet.	This applies only to products for which the labelled posology allows the product to be taken on a daily basis for > 1 month or repeated use for more than 2 days every week. 17 mmol (391 mg) is approximately 20% of the WHO adult recommended maximum daily dietary intake of 2 g sodium and is considered to represent 'high' sodium. This is also relevant for children, where the maximum daily intake is considered to be proportional to adults and based on energy requirements. <Z doses> reflects the lowest number of dosage units for which the threshold of 17 mmol (391 mg) of sodium is reached/ exceeded. Round down to the nearest whole number. For SmPC wording please refer to PRAC recommendation: "*1.3. Sodium-containing effervescent, dispersible and soluble medicines – Cardiovascular events*" (EMA/PRAC/234960/2015).
Sodium laurilsulfate	**09/10/2017** *Corrigendum 19/11/2018*	Cutaneous	Zero	This medicine contains x mg sodium laurilsulfate in each <dosage unit><unit volume> <which is equivalent to x mg/<weight><volume>>. Sodium laurilsulfate may cause local skin reactions (such as stinging or burning sensation) or increase skin reactions caused by other products when applied on the same area.	The thickness of the skin varies considerably according to the body site and with age and can be an important factor in the sensitivity to sodium laurilsulfate (SLS). Sensitivity to SLS will also vary according the type of formulation (and effects of other excipients), the concentration of SLS, contact time and patient population (children, hydration level, skin color and disease). Patient populations with decreased skin barrier functions such as in atopic dermatitis are more sensitive to the irritant properties of SLS.
Sorbic acid (E 200) and salts		Topical	Zero	May cause local skin reactions, (e.g. contact dermatitis).	

Name	Updated on	Route of Administration	Threshold	Information for the Package Leaflet	Comments
Sorbitol (E 420)	**09/10/2017**	Oral, parenteral	Zero	This medicine contains x mg sorbitol in each <dosage unit><unit volume> <which is equivalent to x mg/<weight><volume>>.	The additive effect of concomitantly administered products containing sorbitol (or fructose) and dietary intake of sorbitol (or fructose) should be taken into account. The content of sorbitol in medicinal products for oral use may affect the bioavailability of other medicinal products for oral use administered concomitantly.
Sorbitol (E 420)	**09/10/2017**	Intravenous (IV)	Zero	Sorbitol is a source of fructose. If you (or your child) have hereditary fructose intolerance (HFI), a rare genetic disorder, you (or your child) must not receive this medicine. Patients with HFI cannot break down fructose, which may cause serious side effects. You must tell your doctor before receiving this medicine if you (or your child) have HFI or if your child can no longer take sweet foods or drinks because they feel sick, vomit or get unpleasant effects such as bloating, stomach cramps or diarrhoea.	Patients with hereditary fructose intolerance (HFI) must not be given this medicine unless strictly necessary. Babies and young children (below 2 years of age) may not yet be diagnosed with hereditary fructose intolerance (HFI). Medicines (containing sorbitol/fructose) given intravenously may be life-threatening and should be contraindicated in this population unless there is an overwhelming clinical need and no alternatives are available. A detailed history with regard to HFI symptoms has to be taken of each patient prior to being given this medicinal product.
Sorbitol (E 420)	**09/10/2017**	Oral, parenteral (other than IV)	5 mg/kg/day	Sorbitol is a source of fructose. If your doctor has told you that you (or your child) have an intolerance to some sugars or if you have been diagnosed with hereditary fructose intolerance (HFI), a rare genetic disorder in which a person cannot break down fructose, talk to your doctor before you (or your child) take or receive this medicine.	Patients with hereditary fructose intolerance (HFI) should not take/be given this medicinal product.
Sorbitol (E 420)	**09/10/2017**	Oral	140 mg/kg/day	Sorbitol may cause gastrointestinal discomfort and mild laxative effect.	
Soya oil **Hydrogenated soya oil**		All	Zero	<Medicinal product> contains soya oil. If you are allergic to peanut or soya, do not use this medicinal product.	In line with Arachis oil. SmPC: contraindication.
Stearyl alcohol		Topical	Zero	May cause local skin reactions (e.g. contact dermatitis).	

Name	Updated on	Route of Administration	Threshold	Information for the Package Leaflet	Comments
Sucrose		Oral	Zero	If you have been told by your doctor that you have an intolerance to some sugars, contact your doctor before taking this medicinal product.	SmPC proposal: Patients with rare hereditary problems of fructose intolerance, glucose-galactose malabsorption or sucrase-isomaltase insufficiency should not take this medicine.
Sucrose		Oral	5 g	Contains x g of sucrose per dose. This should be taken into account in patients with diabetes mellitus.	
Sucrose		Oral liquids, lozenges and chewable tablets	Zero	May be harmful to the teeth.	Information to be included only when the medicinal product may be intended for chronic use, e.g. for two weeks or more.
Sulphites including metabisulphites e.g.: Sulphur dioxide (E 220) Sodium sulphite (E 221) Sodium bisulphite (E 222) Sodium metabisulphite (E 223) Potassium metabisulphite (E 224) Potassium bisulphite (E 228)		Oral Parenteral Inhalation	Zero	May rarely cause severe hypersensitivity reactions and bronchospasm.	
Wheat starch (containing gluten)	**09/10/2017** *Corrigendum 19/11/2018*	Oral	Zero	This medicine contains only very low levels of gluten (from wheat starch)<. It is regarded as 'gluten-free'*> and is very unlikely to cause problems if you have coeliac disease. One <dosage unit> contains no more than x micrograms of gluten. If you have wheat allergy (different from coeliac disease) you should not take this medicine. *[* The statement 'gluten-free' applies only if the gluten content in the medicinal product is less than 20 ppm.]*	The name of the excipient on the packaging should be: 'Wheat starch'.
Wool fat (lanolin)		Topical	Zero	May cause local skin reactions (e.g. contact dermatitis).	

Name	Updated on	Route of Administration	Threshold	Information for the Package Leaflet	Comments
Xylitol (E 967)		Oral	10 g	May have a laxative effect. Calorific value 2.4 kcal/g xylitol.	

Appendix: European Union list of fragrance allergens requiring labelling on cosmetic and detergent products

Substance	CAS No
3-Methyl-4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3-buten-2-one	127-51-5
Amyl cinnamal	122-40-7
Amylcinnamyl alcohol	101-85-9
Anisyl alcohol	105-13-5
Benzyl alcohol	100-51-6
Benzyl benzoate	120-51-4
Benzyl cinnamate	103-41-3
Benzyl salicylate	118-58-1
Cinnamal	104-55-2
Cinnamyl alcohol	104-54-1
Citral	5392-40-5
Citronellol	106-22-9
Coumarin	91-64-5
d-Limonene	5989-27-5
Eugenol	97-53-0
Farnesol	4602-84-0
Geraniol	106-24-1
Hexyl cinnamaldehyde	101-86-0
Hydroxycitronellal	107-75-5
Hydroxymethylpentyl-cyclohexenecarboxaldehyde	31906-04-4
Isoeugenol	97-54-1
Lilial	80-54-6
Linalool	78-70-6
Methyl heptine carbonate	111-12-6
Oak moss	90028-68-5
Tree moss	90028-67-4

Corrigendum 1 (19/11/2018)

Phenylalanine, column “Route of Administration”

Rationale: correction of an editorial mistake.

Previous version:

Phenylalanine	Oral

Corrected version:

Phenylalanine	All

Sodium laurilsulfate, column “Name”

Rationale: E number deleted for consistency with the Annex III of Regulation (EC) No 1333/2008 on food additives.

Previous version:

Sodium laurilsulfate (E 487)

Corrected version:

Sodium laurilsulfate

Wheat starch (containing gluten), columns “Information for the Package Leaflet” and “Comments”

Changes and rationale:

Column “Information for the Package Leaflet”: The wording is clarified and consistent with the food regulation (EU) No 828/2014. The statement "gluten-free" relates to the gluten content in the finished medicinal product and not in wheat starch.

Column “Comments”: The first paragraph has been deleted. According to EDQM, there is no correlation between the total protein content and the gluten content. Therefore calculation should be based directly on the batch information about its gluten content.

Previous version:

Wheat starch (containing gluten)	Wheat starch in this medicine contains only very low levels of gluten <regarded as gluten-free*> and is very unlikely to cause problems if you have coeliac disease. One <dosage unit> contains no more than x micrograms of gluten. If you have wheat allergy (different from coeliac disease) you should not take this medicine. *[* The statement “regarded as gluten-free” applies only if the gluten content in wheat starch is less than 20 ppm.]*	In compliance with the Ph. Eur. monograph, the protein limit of 0.3% in wheat starch (total protein test), means that there is no more than 100 ppm (µg/g) of gluten present in the wheat starch. The maximum level of gluten in the excipient can be calculated based on this information (protein content). The name of the excipient on the packaging should be: “Wheat starch”.

Corrected version:

Wheat starch (containing gluten)	This medicine contains only very low levels of gluten (from wheat starch)<. It is regarded as ‘gluten-free’*> and is very unlikely to cause problems if you have coeliac disease. One <dosage unit> contains no more than x micrograms of gluten. If you have wheat allergy (different from coeliac disease) you should not take this medicine. *[* The statement* ’gluten-free’ *applies only if the gluten content in the medicinal product is less than 20 ppm.]*	The name of the excipient on the packaging should be: ’Wheat starch’.

In practice,

- For products < 20 ppm the PIL should state:

"This medicine contains only very low levels of gluten (from wheat starch). It is regarded as 'gluten-free' and is very unlikely to cause problems if you have coeliac disease…."

- For products > 20 ppm the PIL should state:

"This medicine contains only very low levels of gluten (from wheat starch) and is very unlikely to cause problems if you have coeliac disease…"

PRIVILEGED AND CONFIDENTIAL

LITERATURE SEARCH AND REVIEW TOBACCO INGREDIENTS USED BY MANUFACTURERS IN THE PRODUCTION OF CIGARETTES

FINAL REPORT: 2005
For 2004 list of ingredients

Donald E. Gardner PhD, F.ATS
Susan C. Gardner PhD
Inhalation Toxicology Associates
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16	Ethyl butyrate	00105-54-4	22
17	Ethyl decanoate	00110-38-3	22
18	Ethyl hexanoate	00123-66-0	23
19	Ethyl isovalerate	00108-64-5	23
20	Ethyl lactate	00097-64-3	23
21	Ethyl laurate	00106-33-2	23
22	Ethyl myristate	00124-06-1	23
23	Ethyl octanoate	00106-32-1	23
24	Ethyl phenylacetate	00101-97-3	23
25	2-Ethyl-3,(5 or 6)-dimethylpyrazine	27043-05-6 13925-07-0 13360-65-1	23
26	5-Ethyl-3-hydroxy-4-methyl-2(5H)-furanone	00698-10-2	24
27	Hexyl phenylacetate	05421-17-0	24
28	Isoamyl acetate	00123-92-2	24
29	Isobutyl cinnamate	00122-67-8	24
30	Isobutyl phenylacetate	00102-13-6	24
31	alpha-Isobutylphenethyl alcohol (Benzyl isobutyl carbinol) (Benzeneethanol, alpha- (2-	07779-78-4	24

	methylpropyl)-)		
32	Isobutyric acid	00079-31-2	24
33	2-,5-, or 6-Methoxy-3-methylpyrazine	02847-30-5	24
34	2-Methylheptanoic acid	01188-02-9	25
35	2-Methylpyrazine	00109-08-0	26
36	gamma-Octalactone	00104-50-7	29
37	2,3-Pentanedione	00600-14-6	29
38	2-Phenethyl acetate	00103-45-7	29
39	Phenylacetaldehyde	00122-78-1	29
40	Sodium bicarbonate	00144-55-8	29
41	Sucrose octaacetate	00126-14-7	29
42	2,3,5,6-Tetramethylpyrazine	01124-11-4	30
43	Triethyl citrate	00077-93-0	30
44	4-(2,6,6-Trimethylcyclohex-1-enyl)but-2-en-4-one (beta-Damascone)	35044-68-9 23726-91-2	30
	Major Ingredients		30
45	Glycerol	00056-81-5	30
46	Carbon	07440-44-0	31
47	Invert sugar	08013-17-0	38
48	Maple syrup	08029-81-0	38
49	High Fructose Corn Syrup	08029-43-4 977042-84-4	38
50	Corn syrup	08029-43-4	38
51	Cellulose and Cellulose fiber	65996-61-4 09004-34-6	38
52	Sucrose	00057-50-1	38
53	Propylene glycol	00057-55-6	41
54	Brown sugar	00057-50-1	43
55	Honey	08028-66-8	43
56	Menthol and L-Menthol	00089-78-1 02216-51-5	43
57	Potassium carbonate	00584-08-7	47
58	Rum and rum extract	90604-30-1 977089-45-4	47
59	Cocoa, cocoa shells, extract, distillate, powder, alkalized, absolute and tincture	08002-31-1 84649-99-0 68916-17-6 95009-22-6	47
60	Guar gum	09000-30-0	48
61	Prune juice and concentrate	90082-87-4	48
62	Ethyl alcohol, including SDA-4	00064-17-5	48
63	Licorice root, fluid extract and powder	68916-91-6 08008-94-4 97676-23-8	53

64	Graphite	07782-42-5	36
65	Ammonium phosphate dibasic (Diammonium phosphate)	07783-28-0	54
66	Ammonium alginate	09005-34-9	55
67	Chocolate and chocolate liquor		55
68	Lactic acid	00050-21-5 00598-82-3	55
69	Plum juice, concentrate and extract	90082-87-4	55
70	Carob bean gum, absolute and extract	09000-40-2 84961-45-5	55
71	Fig juice concentrate and extract	90028-74-3 68916-52-9	55
72	Sorbitol	00050-70-4	55
73	Ammonium hydroxide	01336-21-6	55
74	Glucose/ Dextrose	00050-99-7 00492-62-6	56
75	Urea	00057-13-6	56
76	Sodium carbonate	00497-19-8	57
77	Fructose	00057-48-7	57
78	Davana oil	08016-03-3	57
79	Lime oil	68916-84-7	57
80	Ethyl 2-methylbutyrate	07452-79-1	57
81	Peppermint oil and absolute and peppermint oil terpeneless	08006-90-4	58
82	Spearmint oil	08008-79-5	58
83	Orange oil and extract (sweet, distilled, terpeneless, and sour/bitter orange oils)	08008-57-9 68606-94-0 68916-04-1	58
84	Molasses extract	08052-35-5	58
85	Coriander extract, seed, and oil	08008-52-4 84775-50-8	58
86	Ethyl vanillin	00121-32-4	58
87	L-Menthone	14073-97-3	58
88	Vanillin	00121-33-5	59
89	Chamomile flower oil, extract and absolute	08002-66-2 08015-92-7	59
	Standard Ingredients		60
90	Acetanisole	00100-06-1	61
91	Acetic acid	00064-19-7	61
92	Acetoin	00513-86-0	61
93	Acetophenone	00098-86-2	61
94	Acetylpyrazine (2-)	22047-25-2	61

95	3-Acetylpyridine (beta-Acetylpyridine)	00350-03-8	61
96	2-Acetylthiazole	24295-03-2	61
97	DL-Alanine, L-Alanine	00302-72-7 00056-41-7	61
98	Alfalfa extract	84082-36-0	61
99	Allyl hexanoate	00123-68-2	62
100	Ammonium alginate	09005-34-9	62
101	Ammonium hydroxide	01336-21-6	62
102	Ammonium phosphate dibasic (Diammonium phosphate)	07783-28-0	62
103	Amyl alcohol	00071-41-0	62
104	Amyl butyrate	00540-18-1	62
105	Amyl formate	00638-49-3	62
106	Amyl octanoate	00638-25-5	62
107	alpha-Amylcinnamaldehyde	00122-40-7	62
108	trans-Anethole	04180-23-8 00104-46-1	63
109	Angelica root extract and oil	84775-41-7	64
110	Anise star oil	08007-70-3	64
111	Anisyl acetate	00104-21-2	64
112	Apple juice concentrate, essence and extract	85251-63-4	64
113	L-Arginine	00074-79-3	64
114	Ascorbic acid	00050-81-7	64
115	L-Aspartic acid	00056-84-8	65
116	Balsam peru and oil	08007-00-9	65
117	Beeswax resinoid and absolute	08006-40-4 08012-89-3	65
118	Beet juice concentrate	89957-90-4	66
119	Benzaldehyde	00100-52-7	66
120	Benzaldehyde glyceryl acetal	01319-88-6	66
121	Benzoic acid	00065-85-0	66
122	Benzoin, resin, resinoid, tincture, gum and absolute	09000-05-9 84012-39-5 09000-72-0	67
123	Benzyl alcohol	00100-51-6	67
124	Benzyl benzoate	00120-51-4	69
125	Benzyl cinnamate (Propenic acid, 3-phenyl, phenylmethyl ester,2-)	00103-41-3	70
126	Benzyl Phenylacetate	00102-16-9	71
127	Benzyl Propionate	00122-63-4	71
128	Bornyl acetate	00076-49-3	71
129	1,3-Butanediol	00107-88-0	71

130	2, 3-Butanedione (Diacetyl)	00431-03-8	71
131	Butanoic acid, 3-methyl-, 4-methylphenyl ester (para-Tolyl 3-methylbutyrate) (p-Tolyl isovalerate)	55066-56-3	71
132	Butter, butter esters, and butter oil	91745-88-9 97926-23-3	71
133	Butyl acetate	00123-86-4	71
134	Butyl alcohol (1-Butanol)	00071-36-3	72
135	Butyl butyryl lactate (butoxy-1-methyl-2-oxoethyl ester butanoic acid, 2-)	07492-70-8	73
136	n-Butyl isovalerate	00109-19-3	73
137	3-Butylidenephthalide	00551-08-6	73
138	Butyric acid	00107-92-6	74
139	Caprylic/Capric triglyceride	65381-09-1	74
140	Caramel and caramel color	08028-89-5	74
141	Carbon	07440-44-0	74
142	Carbon dioxide	00124-38-9	74
143	Cardamom oleoresin, oil, extract, seed oil, and powder	08000-66-6 96507-91-4	76
144	Carob bean gum, absolute and extract	09000-40-2 84961-45-5	77
145	beta-Carotene	07235-40-7	77
146	Carrot oil, seed	08015-88-1	84
147	4-Carvomenthenol	00562-74-3	84
148	beta-Caryophyllene	00087-44-5	84
149	beta-Caryophyllene oxide	01139-30-6	84
150	Cassia bark, buds, oils, and extract	08007-80-5 84961-46-6	84
151	Castoreum, liquid, extract, tincture and absolute	08023-83-4	84
152	Celery seed oil	89997-35-3	84
153	Cellulose and Cellulose fiber	65996-61-4 09004-34-6	84
154	Chamomile flower oil, extract and absolute	08002-66-2 08015-92-7	84
155	Chicory extract	68650-43-1	85
156	Chocolate and chocolate liquor		85
157	1,8-Cineole (Eucalyptol)	00470-82-6	85
158	Cinnamaldehyde	00104-55-2	85
159	Cinnamon bark, buds, leaf, oil, and extract	08015-91-6 08007-80-5	87

160	Cinnamyl acetate	00103-54-8	87
161	Cinnamyl alcohol	00104-54-1	87
162	Cinnamyl cinnamate	00122-69-0	88
163	Citral	05392-40-5	88
164	Citric acid	00077-92-9	92
165	Citronella oil	08000-29-1	92
166	Citronellol	00106-22-9	93
167	Clary sage oil and extract	08016-63-5	93
168	Cocoa, cocoa shells, extract, distillate, powder, alkalized, absolute and tincture	08002-31-1 84649-99-0 68916-17-6 95009-22-6	93
169	Coconut oil	08001-31-8	93
170	Coffee and coffee solid extract	08001-67-0 68916-18-7 84650-00-0	93
171	Cognac white and green oil	08016-21-5	93
172	Coriander extract, seed, and oil	08008-52-4 84775-50-8	93
173	Corn starch	09005-25-8	93
174	beta-Damascone	23726-92-3 23726-91-2	94
175	Davana oil	08016-03-3	94
176	Decanal	00112-31-2	94
177	delta-Decalactone	00705-86-2	94
178	gamma-Decalactone	00706-14-9	94
179	Decanoic acid	00334-48-5	94
180	Diacetyl	00431-03-8	94
181	Diethyl malonate	00105-53-3	94
182	2,3-Diethylpyrazine	15707-24-1	94
183	2,6-Dimethoxyphenol	00091-10-1	95
184	Dimethyl benzyl carbinyl butyrate (alpha, alpha-Dimethylphenethyl butyrate)	10094-34-5	95
185	Dimethyl sulfide	00108-50-9	95
186	3,4-Dimethyl-1,2-cyclopentadione	13494-06-9	95
187	3,7-Dimethyl-1,3,6-octatriene	13877-91-3	95
188	4,5-Dimethyl-3-hydroxy-2,5-dihydrofuran-2-one (3-Hydroxy-4,5-dimethyl-2(5h)furanone)	28664-35-9	95
189	2, 5-Dimethyl-4-hydroxy-3(2h)-furanone (4-Hydroxy-2,5-dimethyl-3(2h)furanone)	03658-77-3	95
190	3,7-Dimethyl-6-octenoic acid	00502-47-6	96

	(Citronellic acid)		
191	alpha,para-Dimethylbenzyl alcohol	00536-50-5	96
192	2,3-Dimethylpyrazine	05910-89-4	95
193	2,5-Dimethylpyrazine	00123-32-0	96
194	Dodecahydro-3A,6,6,9A-tetramethylnaphtho (2,1-B)furan (1,5,5,9-Tetramethyl-13-oxatricyclo(8.3.0.0(4,9))Tridecane)	03738-00-9 06790-58-5	96
195	delta-Dodecalactone	00713-95-1	97
196	gamma-Dodecalactone	02305-05-7	97
197	Ethyl acetate	00141-78-6	97
198	Ethyl alcohol, including SDA-4	00064-17-5	98
199	Ethyl benzoate	00093-89-0	98
200	Ethyl butyrate	00105-54-4	98
201	Ethyl cinnamate (Propenic acid,3-phenyl-,ethyl ester,2-)	00103-36-6	98
202	Ethyl decanoate	00110-38-3	98
203	4-Ethyl guaiacol (4-Ethyl-2-methoxy-phenol)	02785-89-9	98
204	Ethyl heptanoate	00106-30-9	98
205	Ethyl hexanoate (Ethyl caproate)	00123-66-0	98
206	Ethyl isovalerate	00108-64-5	99
207	Ethyl lactate	00097-64-3	99
208	Ethyl laurate	00106-33-2	99
209	Ethyl levulinate	00539-88-8	99
210	Ethyl maltol	04940-11-8	99
211	Ethyl 2-methylbutyrate	07452-79-1	99
212	Ethyl methyl phenylglycidate	00077-83-8	99
213	Ethyl myristate	00124-06-1	99
214	Ethyl nonanoate	00123-29-5	99
215	Ethyl octadecanoate	00111-61-5	100
216	Ethyl octanoate	00106-32-1	100
217	Ethyl oleate	00111-62-6	100
	Ingredients used in mixture studies tobacco smoke studies		100
	Relevant reviews & interesting papers		107

INTRODUCTION

In a continuing effort to improve the safety evaluation of ingredients added to tobacco, this literature review program identifies and reviews relevant abstracts and documents for information regarding potential health effects of a large number of ingredients.

This review is intended to provide an appropriate means for the continuing safety assessment of the ingredients added to tobacco. This is not intended to be a summary of all available data on a particular ingredient; rather, the aim and scope of this review is on providing the sponsors with an overview of available data regarding issues that can play a role in establishing their safe use. Results from this review can aid in (1) prioritizing for additional toxicology testing and for mechanistic studies, (2) facilitating the evaluation of any proposed modifications to cigarettes, and (3) allowing data exchange between the sponsors and the panel members.

A list of 217 ingredients was provided by Covington and Burling as representing the high-priority chemicals. These ingredients are divided into four categories:

1). New ingredients (8). For these ingredients Inhalation Toxicology Associates (ITA) searched the databases for all citations entered into the database since 1965.

2). Major ingredients (45). These are ingredients having a maximum use level (MUL) of 500 ppm or greater. For this category ITA searched the databases for relevant citations between the dates of the last search to 2004.

3). High MUL ingredients (36). This category includes ingredients whose MUL has increased by a factor of 10 or more from the prior year. For these high MUL ingredients ITA searched the databases for relevant citations between the dates of the last search to 2004.

4). Standard ingredients (128) as identified by Covington and Burling. For this category ITA searched the databases for relevant citations between the dates of the last search to 2004.

The first stage involves the collection of relevant data, including the results of *in vivo* and *in vitro* studies. The second stage involves the assessment of these data to determine the acceptability of the study and relevance of the results to the substance as a tobacco ingredient. To meet these objectives, ITA searches the databases using chemical abstract numbers for relevant citations during the dates corresponding to the category in which they are listed. ITA primarily used the American Chemical Society's Chemical Abstract Services and Dialog Database to search for information about ingredients of interest.

A series of databases were used to search for relevant national and international studies. If in the judgment of ITA, the search for a particular ingredient in any of these databases was not expected to produce relevant information, ITA was authorized to omit

the search of such database(s). ITA was also authorized to modify the literature search strategies in order to better meet the needs of the sponsors.

After the sponsors/panel members have had an opportunity to examine this 2004 report and they believe the goals and objectives of this project would benefit by including some "other" sources, ITA would be most willing to expand our coverage to seek out additional publications/reports for any specific ingredient they determine needs more coverage. If it is decided that "other" sources should be added to our list of databases in future years, we would be most pleased to add these sources to our list of databases searched. During 2004, a total of 9427 titles were retrieved of which 461 were identified as potentially relevant and their abstracts were collected and reviewed by ITA. Using the data from these abstracts, a total of 134 full text copies of relevant documents were retrieved by ITA for a more in-depth review.

As in previous years, it is appropriate to establish some generally accepted and recognized criteria that can be used in assessing the toxicological risk of ingredients in a relatively efficient manner. These guidelines are intended to expedite the safety assessment of ingredients added to tobacco. While the material examined was extensive, most of the toxicological testing of ingredients was not designed to evaluate the health hazards of ingredients in cigarette smoke, but instead focused on the hazards associated with exposure to either the pure substances or as additives in some other medium, such as food. This adds to the complexity of trying to interpret and extrapolate this data for assessing and predicting human health risk associated with exposure to those ingredients found in cigarettes. Although many of these studies were not designed to evaluate tobacco additives, the results have to be considered since they aid in providing a complete picture of the database for these chemicals.

From the large number of studies encountered, it was practical to summarize only the most specific and relevant observations. However, situations that have become controversial are dealt with in more detail. While it was appropriate that ITA considered all data and make decisions about the validity and usefulness of these data, certain research areas received lower priority and may have been excluded from further examination. Examples would be studies involving 1) the use of such ingredients in the treatment of a variety of diseases, 2) new methodologies for measurement, 3) studies addressing potential anti-microbial or pesticidal activity, 4) effects reported on plants and lower animal systems and 5) publications not in English. Even with these exclusions, ITA has provided the sponsors with a vast amount of information. Good decisions are most likely to result from integration of all available data, including those demonstrating adverse effects as well as well-designed studies indicting no effects. This was done to provide the sponsors with a broad base of published literature, and they can select from these studies the most relevant information useful in meeting their unique needs. For each ingredient where there was relevant scientific data addressing the safe use of ingredients in cigarette products, these studies are discussed below. All of the titles and abstracts retrieved have been retained, and hard copies of the most relevant papers are available upon request.

In our professional judgment, based on the literature reviewed during this time period, no information has been generated which indicates that the use of the ingredients evaluated in this review presents a hazard to the health of the consumer at the level being used, so far as can be judged by the scientific evidence available.

Thank you for providing ITA the opportunity to review this subject matter and to express an opinion regarding the health effects of these ingredients. We are available to provide further clarification or discussion if you have questions.

Donald E. Gardner PhD., Fellow ATS,
National Associate of the National Academy of Science
Susan C. Gardner PhD.
DATE: February 15, 2006
Inhalation Toxicology Associates, Inc.

INGREDIENTS REVIEW

CATEGORY: NEW INGREDIENTS

PARA-TOLUALDEHYDE
CAS: 104-87-0

Number of relevant papers: 7

GENERAL COMMENTS ON PAPERS LISTED BELOW:
The first five papers listed below provide a broad view of biological activity for a large number (239 to 464) of individual tobacco smoke constituents using an array of short-term assays. The general conclusion reached was that tobacco smoke contains a number of substances that inhibit cell growth using Ascites sarcoma cells, inhibits noradrenaline, stimulated oxidative metabolism in isolated brown fat cells, damages plasma membrane of cultured human lung fibroblasts and may be mutagenic in the Ames test. Although not directly applicable to the human exposure situation, these assays provide information on possible mechanisms involved in the interaction of specific smoke constituents and cell function.

1. Effects of tobacco smoke compounds on the ciliary activity of the embryo chicken trachea in vitro.
Pettersson B, Curvall M, Enzell CR.
Toxicology. 1982;23(1):41-55.

ABSTRACT: The ciliotoxicity of 316 individual compounds representative of the gaseous and semivolatile phases of tobacco smoke has been investigated using chicken tracheal organ cultures. When examined at 5 mM concentration and measuring the time to complete ciliostasis, 36% of the compounds were found to cause ciliostasis within 15 min, while about 50% had no visible effect on the ciliary activity during a 60-min exposure. The majority of the ciliotoxic compounds were either alkylated phenylethers, benzonitriles, benzaldehydes, phenols, benzenes, naphthalenes and indoles, or alpha, beta-unsaturated ketones and aldehydes or C6-C10 aliphatic alcohols, aldehydes, acids and nitriles. Most of the compounds classified as benzoic acids, esters, polyaromatic hydrocarbons, amines and N-heterocycles, except indoles, were found to be inactive.

COMMENTS: Comments are provided above.

2. Effects of tobacco smoke compounds on the noradrenaline induced oxidative metabolism in isolated brown fat cells.

Pettersson B, Curvall M, Enzell CR.
Toxicology. 1980;18(1):1-15.

ABSTRACT: The effect on cell metabolism of 320 individual smoke components have been investigated by measuring their inhibition of noradrenaline induced respiration in isolated hamster brown fat cells. The compounds are representative of the gaseous and semivolatile phases of tobacco smoke. The strongest inhibitors were found within the groups of aliphatic alcohols, aldehydes and acids, of alkylated phenols and indoles and of alpha, beta-unsaturated aliphatic aldehydes and ketones. Some of the aliphatic aldehydes and acids significantly increased the basal respiration of the cells, probably by acting as substrates and/or uncoupling of mitochondrial respiratory control.

COMMENTS: Comments are provided above.

3. Effect of tobacco smoke compounds on the plasma membrane of cultured human lung fibroblasts

Thelestam M, Curvall M, Enzell CR.
Toxicology. 1980;15(3):203-17.

ABSTRACT: The ability of compounds derived from tobacco and tobacco smoke to increase the permeability of the membranes of human lung fibroblasts has been studied by measuring the release of an intracellular marker after short term exposure. Of the 464 compounds tested, about 25% gave rise to severe membrane damage. The most active compounds, when divided according to functionality, were found within the groups of amines, strong acids and alkylated phenols, whereas nitriles and polycyclic aromatic hydrocarbons were found completely inactive. A pronounced effect of the chain length on the activity was observed for the aliphatic alcohols, aldehydes and acids, and all monocyclic aromatic compounds but benzonitriles and benzoic acids showed an increase in activity with increasing alkylsubstitution. It is concluded that tobacco smoke contains a number of membrane damaging substances. These membrane active compounds could not only cause direct toxic reactions but also potentiate the toxic effect by promoting the cell membrane penetration of other toxic substances in tobacco smoke.

COMMENTS: Comments are provided above.

4. Screening of tobacco smoke constituents for mutagenicity using the Ames' test

Florin I, Rutberg L, Curvall M, Enzell CR.
Toxicology. 1980;15(3):219-232.

ABSTRACT: To clarify the mutagenic activity of individual smoke components, 239 compounds, representative of the gaseous and semivolatile phases of tobacco smoke, were assayed for mutagenicity towards 4 histidine-requiring mutants of Salmonella typhimurium (TA 98, TA 100, TA 1535 and TA 1537). All compounds were tested qualitatively both with and without metabolic activation using a liver fraction (S-9) from Aroclor 1254 or methylcholanthrene induced rats. Without S-9, only 2,3-dimethylindole and 2,3,5-trimethylindole showed mutagenic activity that was not enhanced by the

metabolic activation system. 2,6-Diaminotoluene and coronene, which like the above compounds are not documented carcinogens were found to be mutagenic for strain TA 98 with S-9. Mutagenic activity was also observed for the previously known mutagens benz[a]pyrene, chrysene, benz[a]-anthracene, perylene and beta-naphthylamine, on exposure to strains TA 98 and/or TA 100 with S-9.

COMMENTS: Comments are provided above.

5. Effects of tobacco and tobacco smoke constituents on cell multiplication in vitro - CA
Pilotti A, Ancker K, Arrhenius E, Enzell C.
Toxicology. 1975 Sep;5(1):49-62.

ABSTRACT: Ascites sarcoma BP8 cells, cultured in suspension in vitro were used as a general toxicity test system for tobacco and tobacco smoke constituents. Some 250 compounds, representative of these materials, were examined by exposing cells to different concentrations of these constituents and measuring the inhibition of culture growth, which was related to corresponding effects encountered for positive standards. When employing the present cell toxicity test system possible effects of factors such as penetration, distribution and microsomal metabolism of the compounds studied, are not taken into account. The most active constituents were found to be unsaturated aldehydes and ketones, phenols and indoles. The good correlation observed between functional groups and toxicity permits, within the range of functionalities studied, prediction of the toxicity for a compound of known structure.

COMMENTS: Comments are provided above.

6. AMES SALMONELLA/MAMMALIAN MICROSOME MUTAGENICITY TEST AND REVERSE MUTATION ASSAY - E. COLI WP2 UVRA A (STANDARD PLATE TEST AND PREINCUBATION TEST) (OCT. 19, 1988)

Source: EPA/OTS; Doc #86-920000590

ABSTRACT: P-Tolualdehyde (CAS # 104-87-0) was evaluated for mutagenicity in the Ames test (strains TA1535, TA100, TA1537, TA98) with and without metabolic activation (S-9 mix) and in the Escherichia coli (WP2 uvrA) reverse mutation assay at a dose range of 20 ug - 5000 ug/plate in the standard plate test (SPT) and 4 ug - 2500 ug/plate in the preincubation test (PIT). No bacteriotoxic effect was observed with E. coli. Bacteriotoxicity was detected in all Salmonella strains detected at 2500 ug/plate (PIT) and at 5000 ug/plate (SPT). The test substance was determined to be non-mutagenetic.

COMMENTS: P-Tolualdehyde was determined to be non-mutagenic in the Ames test.

7. Naturally occurring carbonyl compounds are mutagens in Salmonella tester strain TA 104
Marnett LJ, Hurd HK, Hollstein MC, Levin DE, Esterbauer H, Ames BN.
Mutat Res. 1985 Jan-Feb;148(1-2):25-34.

ABSTRACT: Strains of Salmonella typhimurium that carry a nonsense mutation at the site of reversion detect a variety of naturally occurring and synthetic carbonyl compounds as direct-acting mutagens. TA104 is reverted efficiently by formaldehyde, alpha, beta-unsaturated aldehydes (enals), and dicarbonyl compounds, such as diacetyl and glutaraldehyde. This strain is much more sensitive to carbonyl mutagenesis than is TA100, a strain previously reported to detect aldehydes as mutagens, or any other characterized strains of Salmonella. Long-chain enals are very toxic to TA104, but addition of a reduced glutathione chase following an incubation period decreases this toxicity, thus enabling the detection of 4-hydroxy-pentenal, a homolog of the lipid peroxidation product, 4-hydroxy-nonenal, as a mutagen. This is the first report of the mutagenicity of a hydroxy-enal, a class of enals produced by lipid peroxidation. Testing conducted with strains that carry the nonsense mutation in different repair backgrounds indicates that the presence of pKM101 and the deletion of the uvrB gene facilitate the detection of enals and dicarbonyls, but not malondialdehyde, as mutagens. Since carbonyl compounds are widely distributed in foods, are generated during cellular metabolism, and are present in body fluids, they may make a significant contribution to the risk of human cancer.

COMMENTS: Additional comments not necessary, abstract satisfactory.

CITRONELLOL
CAS: 106-22-9

Number of relevant papers: 3

1. Effects of fragrance inhalation on sympathetic activity in normal adults

Haze S, Sakai K, Gozu Y.
Jpn J Pharmacol. 2002 Nov;90(3):247-53.

ABSTRACT: We investigated the effects of fragrance inhalation on sympathetic activity in normal adult subjects using both power spectral analysis of blood pressure fluctuations and measurement of plasma catecholamine levels. Fragrance inhalation of essential oils, such 19 Effects of fragrance inhalation on sympathetic activity in normal adults

COMMENTS: This study demonstrated that inhalation of fragrances can stimulate or depress sympathetic activity in human volunteers. While citronellol was not tested, it was identified as being present (27.7%) in rose oil that was tested. Inhaled rose oil significantly inhibited sympathetic activity and decreased adrenaline levels. The authors

suggest that citronellol might be involved in the modulation of sympathetic activity in normal adults.

2. Subchronic inhalation studies of complex fragrance mixtures in rats and hamsters

Fukayama MY, Easterday OD, Serafino PA, Renskers KJ, North-Root H, Schrankel KR.
Toxicol Lett. 1999 Dec 20;111(1-2):175-87.

ABSTRACT: Users of consumer products are invariably and intentionally exposed to complex mixtures in such products. With finished fragrance products, these mixtures may represent 100 or more fragrance raw materials (FRMs). The objective of the described studies was to evaluate the safety of finished fragrance products via the inhalation route. In total, the finished products contained approximately 100 FRMs at concentrations of 1% or greater. Major FRMs evaluated included benzyl acetate, coumarin, hydroxycitronellal, musk ketone, 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethyl-cyclopenta-gamma-2-be nzopyran (HHCB) and phenyl ethyl alcohol. Groups of rats or hamsters were exposed by inhalation (whole body) to the mixtures at 5, 9 or 50 mg/m3 for 4 h per day, 5 days per week for 6 or 13 weeks. For each of the fragrance products, the doses used generally represented a ten- to 100-fold exaggeration of levels expected to be achieved during typical use by consumers. With one exception, the fragrances were aerosolized prior to introduction into the inhalation chamber. The exception product was formulated with a propellant, packaged in a pressurized container and expelled with an automated actuator. In all studies, chamber concentrations of fragrance were monitored. Particle sizes ranged from 0.5 to 7.5 microm, depending on the study. Subchronic exposure to all fragrance mixtures resulted in no toxicologically significant effects on animal survival, behavior, body weights or weight gains, organ weights, or in hematology, clinical chemistry, or urinalysis parameters. No gross pathological or histopathological findings related to test material exposures were observed. These studies support the conclusions that the fragrance mixtures would not pose a hazard to product users based on repeated and exaggerated inhalation exposures of animals.

COMMENTS: Rats and hamsters were exposed by inhalation to a complex mixture of fragrances. The exposure levels were 10 to 100 fold greater than one would expect to be encountered by humans using such fragrances. None of the fragrances produced signs of toxicity following exposures up to 13 weeks. No histopathological abnormalities were reported in trachea or lungs. The results are consistent with those earlier studies of Gaworski 1998 that exposed rats to mixtures of flavor ingredients in a subchronic smoke inhalation study.

3. Fragrance compounds and essential oils with sedative effects upon inhalation

Buchbauer G, Jirovetz L, Jager W, Plank C, Dietrich H.
J Pharm Sci. 1993 Jun;82(6):660-4.

ABSTRACT: Fragrance compounds and essential oils with sedative effects influence the motility of mice in inhalation studies under standardized conditions. A significant drop in the motility of mice was registered following exposure to these fragrances. The same results were achieved when the mice were artificially induced into overagitation by intraperitoneal application of caffeine and subsequently subjected to inhalation of fragrance compounds and essential oils. These results proved the sedative effects of these fragrants via inhalative exposure in low concentrations. Blood samples were taken from the mice after a 1-h inhalation period. Chromatographic and spectroscopic methods were used to detect and characterize the actual effective compounds after solid-phase extraction. Serum concentrations of 42 different substances, including fragrance compounds, were found in low ranges (ng/mL serum). The results contribute to the correct interpretation of the term aromatherapy (i.e., a stimulating or sedative effect on the behaviour of individuals only upon inhalation of fragrance compounds).

COMMENTS: A one-hour inhalation of citronellol showed a significant sedative effect in over-agitated (caffeine-treated) mice but not with animals without prior caffeine induction. These sedative effects were observed at low blood concentrations (2.0 ng/mL). Substances that produce such an effective may interact with lipids of cell membranes in the cortex thus indicating a direct pharmacological interaction of fragrance molecules with bodily tissue.

ETHYL HEPTANOATE
CAS: 106-30-9

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ISOAMYL FORMATE
CAS: 110-45-2

Number of relevant papers: 2

1. Mutagenic and antimutagenic activities of flavoring agents used in foodstuffs -

Yoo, Y.S. (1986)
Osaka-shi Igakkai Zasshi [J. Osaka City Medical Center], 34(3-4), 267-288

ABSTRACT: N/A

COMMENTS: This article was in Japanese and was not translated. Briefly, these investigators tested for genotoxicity in 33 synthetic flavorings used in foodstuffs. Isoamyl formate had little or no toxic effect and was considered to be negative in the assay system.

2. Primary mutagenicity screening of food additives currently used in Japan –

Ishidate, M; Sofuni, T; Yoshikawa, K;
Food Chem Toxicol 22:623-636.

ABSTRACT: Salmonella/microsome tests (Ames tests) and chromosomal aberration tests in vitro using a Chinese hamster fibroblast cell line were carried out on 190 synthetic food additives and 52 food additives derived from natural sources, all of which are currently used in Japan. Fourteen out of 200 tested in the Ames assay showed positive effects and 54 out of 242 were positive in the chromosome test. Three additives (erythorbic acid, chlorine dioxide and beet red) were positive only in the Ames test, although their mutagenic potentials were relatively weak, while 43 additives were positive only in the chromosome test. Eleven additives (calcium hypochlorite, cinnamic aldehyde, L-cysteine monohydrochloride, Food Green No. 3 (Fast Green FCF), hydrogen peroxide, potassium bromate, sodium chlorite, sodium hypochlorite, sodium nitrite, cacao pigment and caramel) were positive in both the Ames test and the chromosome test. The usefulness of such primary screening tests combining two different genetic end-points, gene mutation and chromosomal aberration, and some correlation between mutagenicity and carcinogenicity of food additives are discussed.

COMMENTS: These investigators did primary screening of over 200 food additives using both the Ames test and chromosomal aberration tests. Only a few (11) were positive in both tests. More additives were positive in the chromosome test than the Ames test indicating that chromosomal aberrations can be induced by a wider range of additives than the Ames test, and suggesting that this may indicate not only initiators of carcinogenesis but also promoters. It should be recognized that data from such short term *in vitro* tests needs further *in vivo* testing to predict carcinogenicity. The correlation between carcinogenicity and mutagenicity of additives are discussed.

HEXYL ACETATE
CAS: 142-92-7

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

PECTIN
CAS: 9000-69-5

Number of relevant papers: 3

1. Pectin and cashew nut allergy: Cross-reacting allergens?

Rasanen L, Makinen-Kiljunen S, Harvima RJ.
Allergy. 1998 Jun;53(6):626-8.

ABSTRACT: N/A

COMMENTS: This case report indicates that exposure to pectin may cause sneezing, rhinitis, conjunctivitis and contact urticaria. Occupational sensitization with rhinitis and asthma from pectin has been previously identified. In this study, blood basophil histamine release and serum IgE were positive.

2. Occupational asthma caused by pectin inhalation during the manufacture of jam

AJ Cohen, MS Forse and SM Tarlo
Chest, Vol 103, 309-311, Copyright © 1993

ABSTRACT: We report a case of pectin-induced occupational asthma in a 35-year-old man. His job involved mixing powdered pectin into a fruit puree during the manufacture of jam. Within minutes of adding pectin, he developed coryza, rhinorrhea, coughing, and wheezing. His symptoms cleared during weekends while away from work and improved with the use of a protective facemask at work. Peak flow rates were significantly lower while at work compared with those at home, and a prick skin test with the pectin powder was positive. We conclude that pectin should be added to the list of the substances known to induce occupational asthma.

COMMENTS: This is another case report describing a pectin-induced occupational asthma. The individual exhibited positive skin testing to pectin. The authors suggested that pectin should be considered to be an allergen that causes occupational asthma.

3. Bacterial mutagenicity testing of 49 food ingredients gives very few positive results.

Prival MJ, Simmon VF, Mortelmans KE.
Mutat Res. 1991 Aug; 260(4):321-9.

ABSTRACT: 49 substances permitted for use in food in the United States was tested for mutagenicity in the Ames Salmonella typhimurium assay and in Escherichia coli strain WP2. Four of these substances caused increases in revertant counts in S. typhimurium. Two of these four (papain and pepsin) were found to contain histidine, and therefore the results of the tests on these two substances could not be taken as demonstrating mutagenicity. The other two substances causing increases in revertant counts (hydrogen peroxide and potassium nitrite) were mutagenic. The results on one chemical, beta-carotene, were evaluated as inconclusive or questionable. The remaining 44 substances were nonmutagenic in the test systems used. It is concluded that, for those generally physiologically innocuous chemicals tested, there are very few 'false positives' in the bacterial test systems used.

COMMENTS: The Salmonella Ames test and E coli mutagenicity assays were used to evaluate the mutagenicity of a number of food ingredients. Pectin gave no evidence of

mutagenicity. In these studies, the frequency of positive results was much lower than in many other previous studies. The authors believe that this is due to the fact that the chemicals tested were almost all nontoxic to mammals and to bacteria even at relatively high doses.

CORN STARCH
9005-25-8

Number of relevant papers: 2

1. Inhaled cornstarch glove powder increases latex-induced airway hyper-sensitivity in guinea-pigs

Barbara J.; Santais M.-C.1; Levy D.A.2; Ruff F.1; Leynadier F.
Clinical & Experimental Allergy 34 (6): 978-983

ABSTRACT: Summary Background. Breathing is one of the most important modes of sensitization to natural rubber latex (NRL) for health-care workers, a group most at risk. Cornstarch powder (CSp) from medical powdered NRL gloves is known to be an allergen carrier, and sensitization to NRL can occur by inhaling airborne particles from such gloves. Objective: The aim of this study was to demonstrate, using an experimental model, which CSp may act as an adjuvant in NRL-induced airway hyper-responsiveness. Methods: Guinea-pigs were exposed to aerosolized NRL-contaminated CSp or to NRL in saline solution for 1 h every day for 2 weeks. The control groups were exposed either to CSp or to saline alone. An additional group of guinea-pigs was exposed to aerosolized ovalbumin (OVA) in saline. Three weeks after the last exposure, specific bronchial challenges were performed. In addition, Specific IgG and IgG1 in sera and thromboxane (Tx) B2 levels in bronchoalveolar lavage fluid (BALF) were measured. Results: The NRL challenge caused significant bronchospasm in the animals that had been exposed to NRL compared with those in the control groups ($P<0.02$). Guinea-pigs exposed to OVA also demonstrated a significant bronchospasm after OVA challenge ($P<0.001$). The guinea-pigs that had inhaled NRL-contaminated CSp had a significantly higher bronchoconstriction level than those that had inhaled NRL alone ($P<0.02$). Specific IgG and IgG1 were undetectable in sera from all groups, whereas significant amounts of TxB2 ($P<0.001$) were found in the lungs of the guinea-pigs exposed to NRL or OVA. Conclusion: Inhaling CSp increases the airway response to NRL. The fact that specific IgG and IgG1 were not detected might be the result of an immune response limited to the airways. This finding is supported by a significant increase of TxB2 level in the BALF of sensitized guinea-pigs.

COMMENTS: These authors previously reported that corn starch acts as an immunoadjuvant in guinea pigs that were previously sensitized to rubber latex by the IP route. This experimental model was used to determine if corn starch potentiates immunotoxicity of rubber latex through inhaling latex adsorbed onto corn starch. While the direct relevance of this study was to the health care workers who become sensitive to

rubber latex, it also indicates that breathing corn starch may induce hypersensitivity and may act as an adjuvant, resulting in increased airway responsiveness.

2. Bronchial provocation testing in the diagnosis of occupational asthma due to latex surgical gloves –

G Pisati, A Baruffini, F Bernabeo, and R Stanizzi
Eur Respir J 1994; 7: 332-336

ABSTRACT: In sensitized subjects, provocation tests to latex may induce severe systemic reactions and even anaphylactic shock. It is probable that part of the risk is due to the difficulty in grading the stimulating dose and in starting from very low levels of exposure. To identify the aetiological agent of work-related asthma in four nurses with previous allergic contact urticaria to latex surgical gloves dusted with cornstarch powder, we performed a specific bronchial provocation test study, based on exposure on three different days to nonpowdered latex surgical glove extract, powdered latex surgical glove extract and cornstarch powder extract, respectively. Extracts were nebulized in increasing concentrations in a 7 m3 challenge room, in the absence of the patients. The initial extract concentration was a tenfold dilution of the predetermined skin test end-point in the individual undergoing challenge, and the highest concentration was the undiluted extract. After exposure, the patients' forced expiratory volume in one second (FEV1) was monitored for 2 h. If FEV1 decreased by at least 15%, the next scheduled exposure was not carried out and FEV1 was monitored over a period of 24 h. Whereas nebulization of cornstarch powder extract caused no bronchial reaction in the patients, nebulization of nonpowdered latex surgical glove extract induced immediate bronchoconstriction in two subjects as an undiluted solution, and nebulization of powdered latex surgical glove extract induced immediate bronchoconstriction in all subjects at the 1:10 dilution. No systemic reaction was elicited by the bronchial provocation challenges. Our results demonstrate that airborne powder from latex gloves can be an inhalative occupational hazard. Latex, absorbed by the cornstarch powder and then airborne when gloves were handled, was the causative agent of the respiratory events in our patients. The standardized method that we used minimizes the risk of eliciting systemic reactions when performing specific bronchial provocation tests to latex.

COMMENTS: This paper describes the use of skin tests and specific bronchial challenge to determine the causative agent of asthma in four hospital nurses. The nurses were experimentally exposed to cornstarch powder alone and in combination with latex glove extract. The skin tests with powdered latex surgical gloves extract gave a positive reaction from the 1:100 dilution, whereas pure cornstarch powder did not induce any reaction. The results of the bronchial provocation test similarly demonstrated that latex was the causative agent of asthma in these patients, since bronchoconstriction was observed after the challenges with powdered and unpowdered glove extract, but not after the cornstarch powder extract alone.

L-MENTHONE
14073-97-3

Number of relevant papers: 1

1. Inhibition of Human Liver Microsomal (S)-Nicotine Oxidation by (-)-Menthol and Analogues

MacDougall JM, Fandrick K, Zhang X, Serafin SV, and Cashman JR
Chem Res Toxicol 16: 988-993

ABSTRACT: Menthol is a widely used flavoring ingredient present in mouthwash, foods, toothpaste, and cigarettes; yet, the pharmacological effects of menthol have not been widely studied. Mentholated cigarette smoking may increase the risk for lung cancer. Many African American smokers smoke mentholated cigarettes, and African Americans have a significantly higher incidence of lung cancer as compared with whites. There may be a relationship between the incidence of lung cancer and the type of cigarette smoked because the use of mentholated cigarettes by white smokers is significantly less and the incidence of lung cancer is less. The mechanism whereby (-)-menthol could increase the health risk of smoking is not known. The results of our in vitro studies herein show that (-)-menthol and synthetic congeners inhibit the microsomal oxidation of nicotine to cotinine and the P450 2A6-mediated 7-hydroxylation of coumarin. Replacement of the alcohol oxygen atom of menthol with other heteroatoms increased the potency of P450 2A6 inhibition. Thus, the K(i) value of (-)-menthol for inhibition of microsomal nicotine oxidation was 69.7 micro M but neomenthyl thiol possesses a K(i) value of 13.8 micro M. Menthylamine inhibited nicotine oxidation with a K(i) value of 49.8 micro M, but its hydroxylamine derivative gave an IC(50) value of 2.2 micro M. A series of 16 menthol derivatives and putative metabolites were procured or chemically synthesized and tested as inhibitors of P450 2A6. While highly potent inhibition of P450 2A6 was not observed for the menthol analogues examined, it is nevertheless possible that smoking mentholated cigarettes leads to inhibition of nicotine metabolism and allows the smoker to achieve a certain elevated dose of nicotine each day. This may be another example of self-medication to obtain the desired effect of nicotine.

COMMENTS: Abstract summary of the paper is adequate.

CATEGORY: HIGH MUL'S INGREDIENTS

ACETIC ACID
CAS: 64-19-7

Number of relevant papers: 2

1. On the deposition of volatiles and semivolatiles from cigarette smoke aerosols: Relative rates of transfer of nicotine and ammonia from particles to the gas phase

Seeman Jeffrey I,; Lipowicz Peter J; Piade Jean-Jacques; Poget Laurent; Sanders Edward B; Snyder James P; Trowbridge Clarence G
Chemical Research in Toxicology , Volume: 17 , Number: 8 , Page: 1020-1037

ABSTRACT: The hypothesis that elevated levels of ammonia-releasing compounds in tobacco and ammonia in mainstream (MS) smoke increase the rate and amount of nicotine evaporation from the particles of MS smoke aerosol was examined by kinetic modeling and experiments with MS cigarette smoke. Computational simulation of a kinetic mechanism describing volatile loss of nicotine, ammonia, and acetic acid from an aqueous solution was used to compute the time-dependent concentration of all species in the model. Because of the high volatility of ammonia relative to that of nicotine, variation over a wide range of initial ammonia concentration had no significant effect upon the rate of loss of nicotine from the model system. The effects of a variation in the volatile loss rate constant for ammonia and for the acid were examined. The simulations show that ammonia is lost from the model solution at a greater rate than nicotine and acid, and the loss of volatile acid has a significant role in the rate and amount of nicotine loss. Simulations with a model system undergoing a continuous steady addition of ammonia showed that high rates of ammonia addition could significantly increase the rate of nicotine volatile loss from the model solution. A series of smoking experiments was performed using blended cigarettes connected to a denuder tube. Deposition of smoke constituents can occur directly from the gas phase and by the deposition of smoke aerosol particles themselves. As nicotine exists >99% in the particle phase of MS smoke, in the absence of particle deposition, denuder tube deposition of nicotine occurs via the evaporation-deposition pathway. Solanesol, a nonvolatile tobacco and smoke terpene, was used to quantify the amount of particle deposition onto the denuder tube. The amount of ammonia deposited on the denuder tube was an order of magnitude greater than that of nicotine, showing that ammonia evaporates from the MS smoke particles much faster than does nicotine. The experimental results were supported and explained by the aqueous model simulations. Included in these experiments are cigarettes that differ in their MS smoke ammonia content by a factor of ca. five. However, an increased amount of MS smoke ammonia does not increase the rate of nicotine loss from the particles. The combined results support the conclusion that ammonia in mainstream smoke has little effect, if any, upon the rate and amount of nicotine evaporation from MS smoke particles.

COMMENTS: A computation model using chemical kinetics was employed to exam the role of volatile acids (acetic acid or formic acid) and bases (ammonia) in nicotine evaporation from smoke aerosol particles. Experimental results and model simulations indicate that ammonia and acetate evaporate from particles far faster than nicotine. Ammonia in mainstream smoke aerosol has little effect on nicotine loss in smoke particles. Increasing acid volatility increased the rate and amount of nicotine and ammonia loss. Formic acid caused a similar but slower effect than acetic acid. This paper is relevant to the effects of acetic acid as an ingredient in cigarette smoke in that it describes the theoretical effect of acetic acid on nicotine and ammonia volatility.

2. Physician diagnosed asthma, respiratory symptoms, and associations with workplace tasks among radiographers in Ontario, Canada

G M Liss, S M Tarlo, J Doherty, J Purdham, J Greene, L McCaskell, M Kerr
Occup Environ Med 2003; 60:254–261.

ABSTRACT: Background: Medical radiation technologists (MRTs) or radiographers have potential exposure to chemicals including sensitisers and irritants such as glutaraldehyde, formaldehyde, sulphur dioxide, and acetic acid. Aims: To determine the prevalence of asthma and work related respiratory symptoms among MRTs compared with physiotherapists, and to identify work related factors in the darkroom environment that are associated with these outcomes. Methods: As part of a two component study, we undertook a questionnaire mail survey of the members of the professional associations of MRTs and physiotherapists in Ontario, Canada, to ascertain the prevalence of physician diagnosed asthma, and the prevalence in the past 12 months of three or more of the nine respiratory symptoms (previously validated by Venables et al to be sensitive and specific for the presence of self reported asthma). Information on exposure factors during the past 12 months, such as ventilation conditions, processor leaks, cleanup activities, and use of personal protective equipment was also collected. Results: The survey response rate was 63.9% among MRTs and 63.1% among physiotherapists. Most analyses were confined to 1110 MRTs and 1523 physiotherapists who never smoked. The prevalence of new onset asthma (since starting in the profession) was greater among never smoking MRTs than physiotherapists (6.4% v 3.95%), and this differed across gender: it was 30% greater among females but fivefold greater among males. Compared with physiotherapists, the prevalence of reporting three or more respiratory symptoms, two or more work related, and three or more work related respiratory symptoms in the past 12 months was more frequent among MRTs, with odds ratios (ORs) (and 95% confidence intervals) adjusted for age, gender, and childhood asthma, of 1.9 (1.5 to 2.3), 3.7 (2.6 to 5.3), and 3.2 (2.0 to 5.0), respectively. Analyses examining latex glove use indicated that this was not likely to account for these differences. Among MRTs, respiratory symptoms were associated with a number of workplace and exposure factors likely to generate aerosol or chemical exposures such as processors not having local ventilation, adjusted OR 2.0 (1.4 to 3.0); leaking processor in which clean up was delayed, 2.4 (1.6 to 3.5); floor drain clogged, 2.0 (1.2 to 3.2); freeing a film jam, 2.9 (1.8 to 4.8); unblocking a blocked processor drain, 2.4 (1.6 to 3.7); and cleaning up processor chemical spill, 2.8 (1.9 to 4.2). These outcomes were not associated with routine tasks unlikely to generate exposures, such as working

outside primary workplace, loading film into processor, routine cleaning of processors, or removing processed film. Males reported that they carried out a number of tasks potentially associated with irritant exposures more frequently than females, consistent with the marked increase in risk for new onset asthma. Conclusions: These findings suggest an increase of work related asthma and respiratory symptoms shown to denote asthma among MRTs, which is consistent with previous surveys. The mechanism is not known but appears to be linked with workplace factors and may involve a role for irritant exposures.

COMMENTS: This study described a higher prevalence of asthma and work-related respiratory symptoms among medical radiation technologists as compared to other workers (physiotherapists) and attempted to identify environmental factors associated with these outcomes. Medical radiation technologists are exposed to acetic acid and other chemicals during the processing of films, however, the causative agent(s) in these work-related respiratory symptoms is currently unknown.

BENZALDEHYDE
CAS: 100-52-7

Number of relevant papers: 2

1. The GreenScreen genotoxicity assay: a screening validation programme -

Cahill PA, Knight AW, Billinton N, Barker MG, Walsh L, Keenan PO, Williams CV, Tweats DJ, Walmsley RM.
Mutagenesis. 2004 Mar;19(2):105-19

ABSTRACT: A yeast (Saccharomyces cerevisiae) DNA repair reporter assay termed the GreenScreen assay (GSA) is described. This is a novel, cost-effective genotoxicity screen, developed to provide a pre-regulatory screening assay for use by the pharmaceutical industry and in other applications where significant numbers of compounds need to be tested. It provides a higher throughput and a lower compound consumption than existing eukaryotic genotoxicity assays and is sensitive to a broad spectrum of mutagens and, importantly, clastogens. We describe a simple, robust assay protocol and a validation study. The end-point of the test reflects the typically eukaryotic chromosomes and DNA metabolizing enzymes of yeast. The capacity for metabolic activation (MA) in yeast is limited compared with the mammalian liver or its extracts, but the assay does detect a subset of compounds that would require MA in existing genotoxicity tests. The GSA detects a different spectrum of compounds to bacterial genotoxicity assays and thus, together with an in silico structure-activity relationship (SAR) screen, and possibly a high throughput bacterial screen, would provide an effective preview of the regulatory battery of genotoxicity tests.

COMMENTS: This paper describes a genotoxicity assay that measures a different end-point (DNA repair induction) using a different type of cell (yeast) than the Ames test. The

authors used this yeast assay to test over 100 compounds. In this assay benzaldehyde was positive for genotoxicity.

2. Effects of garage employment and tobacco smoking on breathing-zone concentrations of carbonyl compounds.

Zhang L; Chung FL; Boccia L; Colosimo S; Liu WL; Zhang JF.
AIHA Journal 64(3): 388-393, 2003. (26 refs.)

ABSTRACT: Exposure to carbonyl compounds may cause adverse health effects. The present study examined whether working in a garage and smoking can significantly affect personal "daily" exposure to a number of important carbonyl compounds. The study was carried out on 37 subjects including 22 garage workers (9 smokers and 13 nonsmokers) and 15 nongarage workers or so-called controls (4 smokers and 11 nonsmokers). Daily exposure was estimated using 48-hour integrated measurement of breathing-zone concentrations. The measurement involved the use of a passive carbonyl sampler and high performance liquid chromatography/fluorescence analysis technique. Each subject was measured for up to three measurement sessions. A wide range of breathing-zone concentrations (unit: microgram per cubic meter) was observed for each of the following carbonyls: formaldehyde (14.1-80.1); acetaldehyde (8.41-80.3); acetone (0.65-1096); acrolein (<0.14-3.71); propionaldehyde (1.08-14.6); crotonaldehyde (<0.13-2.80); benzaldehyde (1.79-9.91); and hexaldehyde (0.122-22.4). Statistical significance of smoking effects and working in a garage effects were assessed using SAS mixed models. The results show that the garage workers had significantly higher levels of formaldehyde and acetal dehyde than the controls, and that the smokers had significantly higher levels of acetaldehyde, propionaldehyde, and hexaldehyde, than the nonsmokers (P<.10). Garage employment and smoking appeared to increase breathing-zone concentrations of crotonaldehyde. In general, within-subject variations were smaller than between-subject variations on 48-hour averaged breathing-zone concentrations of carbonyl compounds.

COMMENTS: While the primary focus of this study was to determine exposure to 8 carbonyl compounds commonly found in a garage environment, they also examined the added effect of smoking on breathing zone concentration of these carbony compounds. While all carbonyls tested are known to be present in tobacco smoke, only acetaldehyde, propionaldehyde and hexaldehyde were found to be higher in the workers' breathing zone area of smokers as compared to nonsmokers.

BUTYRIC ACID
CAS: 107-92-6

Number of relevant papers: 1

1. Oncogenic Ras promotes butyrate-induced apoptosis through inhibition of gelsolin expression

Lidija Klampfer, Jie Huang, Takehiko Sasazuki, Senji Shirasawa, and Leonard Augenlicht
J. Biol. Chem., Vol. 279, Issue 35, 36680-36688

ABSTRACT: Activation of Ras promotes oncogenesis by altering a multiple of cellular processes, such as cell cycle progression, differentiation, and apoptosis. Oncogenic Ras can either promote or inhibit apoptosis, depending on the cell type and the nature of the apoptotic stimuli. The response of normal and transformed colonic epithelial cells to the short chain fatty acid butyrate, a physiological regulator of epithelial cell maturation, is also divergent: normal epithelial cells proliferate, and transformed cells undergo apoptosis in response to butyrate. To investigate the role of k-ras mutations in butyrate-induced apoptosis, we utilized HCT116 cells, which harbor an oncogenic k-ras mutation and two isogenic clones with targeted inactivation of the mutant k-ras allele, Hkh2, and Hke-3. We demonstrated that the targeted deletion of the mutant k-ras allele is sufficient to protect epithelial cells from butyrate-induced apoptosis. Consistent with this, we showed that apigenin, a dietary flavonoid that has been shown to inhibit Ras signaling and to reverse transformation of cancer cell lines, prevented butyrate-induced apoptosis in HCT116 cells. To investigate the mechanism whereby activated k-ras sensitizes colonic cells to butyrate, we performed a genome-wide analysis of Ras target genes in the isogenic cell lines HCT116, Hkh2, and Hke-3. The gene exhibiting the greatest down-regulation by the activating k-ras mutation was gelsolin, an actin-binding protein whose expression is frequently reduced or absent in colorectal cancer cell lines and primary tumors. We demonstrated that silencing of gelsolin expression by small interfering RNA sensitized cells to butyrate-induced apoptosis through amplification of the activation of caspase-9 and caspase-7. These data therefore demonstrate that gelsolin protects cells from butyrate-induced apoptosis and suggest that Ras promotes apoptosis, at least in part, through its ability to down-regulate the expression of gelsolin.

COMMENTS: This paper indicates a possible butyrate effect, but is not directly relevant to inhaled ingredient.

CAPRYLIC/CAPRIC TRIGLYCERIDE
CAS: 65381-09-1

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

BETA-CARYOPHYLLENE OXIDE
CAS: 1139-30-6
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

GAMMA-DECALACTONE
CAS: 706-14-9
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

2,5-DIMETHYLPYRAZINE
123-32-0

Number of relevant papers: 3

1. Pyrazine derivatives in cigarette smoke inhibit hamster oviductal functioning –

Karen Riveles, Ryan Roza, Janet Arey and Prue Talbot
Reproductive Biology and Endocrinology 2004, 2:23 doi:10.1186/1477-7827-2-23

ABSTRACT: Our past studies have shown that cigarette smoke inhibits oviductal functioning in vivo and in vitro. The goals in this study were to identify pyrazine derivatives in cigarette smoke solutions that inhibit ciliary beat frequency, oocyte pickup rate, and infundibular smooth muscle contraction in the hamster oviduct and to determine their lowest observable adverse effect levels (LOAELs) using in vitro bioassays.
Methods: MS smoke solutions were fractionated using solid phase extraction cartridges and the fractions were both tested on the hamster oviduct in vitro and analyzed by gas chromatography-mass spectrometry to identify individual pyrazine derivatives. Commercial pyrazine standards were purchased, assayed for purity, and tested in dose-response studies on hamster oviducts. The LOAEL and efficacy were determined for each compound in the in vitro bioassays. Statistical significance was determined using the Student's t-Test where $p < 0.05$. Results: The LOAELs for the most inhibitory pyrazine derivatives in the ciliary beat frequency, oocyte pickup rate, and infundibular smooth muscle contraction assays were as follows: for pyrazine (1 picomolar, 10 picomolar, and 1 nanomolar); for 2-methylpyrazine (1 picomolar, 10 picomolar, and 10 picomolar); and for 2-ethylpyrazine (1 picomolar, 10 picomolar, and 1 picomolar). Six of the seven pyrazine derivatives tested (pyrazine, 2-methylpyrazine, 2-ethylpyrazine, 2-methoxy-3-methylpyrazine, 2,5-dimethylpyrazine, and 2,3,5-trimethylpyrazine) were inhibitory in picomolar or nanomolar doses in all three bioassays, while the seventh derivative, 2,6-dimethylpyrazine, had LOAELs in the nanomolar to micromolar range.
Conclusion: This work shows that very low doses of pyrazines significantly inhibit proper oviductal functioning, raising questions regarding the safety of these compounds in cigarettes and other consumer products.

COMMENTS: An *in vitro* assay was used to study the effects of pyrazine and its derivatives in cigarette smoke on hamster oviductal functioning. Pyrazine derivatives had

equal or greater effects than pyrazine and inhibited ciliary beating, oocyte pickup rate and smooth muscle contraction in hamster oviductal explants at nanomolar and picomolar doses. Oocyte pickup rate was the most sensitive parameter, and derivatives with single ethyl or methyl substitutions were among the most inhibitory. The LOAEL of 2,5-dimethylpyrazine was equal to pyrazine for oocyte pickup rate (10^{-11} M) and smooth muscle contraction (10^{-9} M), but 10,000 times greater for ciliary beat frequency (10^{-8} M). For all three measurements, 2,5-dimethylpyrazine was more potent than 2,6-dimethylpyrazine. The authors suggested that these data concur with results reported from *in vivo* hamster studies and epidemiological studies that show increased risk of ectopic pregnancies and spontaneous abortions in female smokers. The authors recommend that further toxicological testing of pyrazines be conducted.

2. The influence of cigarette moisture to the chemistry of particulate phase smoke of a common commercial cigarette -

Q. Zha and S.C. Moldoveanu
Beiträge zur Tabakforschung International/Contributions to Tobacco Research Volume 21(3):184-191

ABSTRACT: This study presents the results on the influence of cigarette moisture content to the chemical composition of particulate phase smoke. Seventy-five selected compounds were monitored for the comparison of particulate phase smoke of a commercial full-flavored (FF) cigarette with three different moisture contents at 7.8%, 14.5% and 20.4%, respectively. It was demonstrated that the smoke of a dry cigarette is richer in lower molecular mass compounds than a regular cigarette. On the other hand, the smoke of a moist cigarette is richer in higher molecular mass compounds than a regular cigarette. To maximize the influence of cigarette moisture to the chemical composition, a separate set of measurements were done using only the first three puffs of smoke. The accumulation of moisture in the tobacco column of a burning cigarette may influence the smoke composition, as generated during burning. The differences between dry, regular and moist cigarettes were more obvious for the first three puffs.

COMMENTS: While this is not a health effect study, the results are interesting. These investigators compared the chemical composition of cigarette smoke from cigarettes with three moisture levels (dry-8.3%, regular-11.6%, and moist- 12.9%). The first three puffs showed the greatest differences. The nicotine content and total particulate matter (TPM) was reduced with increasing moisture. The data would indicate that the dry cigarette had a higher percentage of semi-volatile compounds in TPM. The data presents additional evidence that the cigarette moisture content significantly affects the chemistry of the particulate phase of smoke. Of the 75 compounds tested, the more volatile compounds were more affected than the less volatile compounds. Compared to the first three puffs, the particulate phase of smoke from the entire cigarette was less sensitive to the moisture content.

3. Identification of compounds in cigarette smoke that inhibit hamster oviductal functioning. -

K. Riveles, R. Roza and P. Talbot. Cell Biology & Neuroscience, UC Riverside, Riverside, CA. Poster SETAC Utah 2003.

ABSTRACT: Our past studies have shown that chemicals in cigarette smoke inhibit oviductal functioning in vivo and in vitro. The purposes of this study were to identify the individual toxicants in cigarette smoke solutions that inhibit oocyte pickup rate, ciliary beat frequency, and infundibular smooth muscle contraction and to determine their effective doses using in vitro bioassays. Solid phase extraction and gas chromatography-mass spectrometry were used to identify individual chemicals in the mainstream and sidestream cigarette smoke solutions that were active in the above assays. Pyridines, pyrazines, indoles, quinolines, and phenols were identified in the solutions of mainstream and sidestream cigarette smoke. Commercially available standards of the identified compounds were purchased, assayed for purity, and tested in dose-response studies on hamster oviducts. The lowest observable adverse effect level and efficacy were determined for each compound using the oocyte pickup rate, ciliary beat frequency, and infundibular muscle contraction assays. Previously, we have shown that several pyridine compounds including 2-methylpyridine, 4-methylpyridine, 2-ethylpyridine, 3-ethylpyridine, and 4- vinylpyridine were inhibitory at picomolar concentrations in all three bioassays. Further studies have shown that compounds in the pyrazine group: 2- methylpyrazine, ethylpyrazine, 2-methoxy-3-methylpyrazine, 2, 5-dimethylpyrazine, and 2, 3, 5-trimethylpyrazine, were inhibitory in pico or nanomolar doses. Both quinoline and isoquinoline were inhibitory in picomolar doses. 5-Methylindole showed inhibition in the nanomolar range. Indole, which is found in large quantities relative to other compounds in the smoke, showed inhibition at 10-15M. The phenolic compounds were not as inhibitory as the other classes of compounds in the bioassays, although hydroquinone and 4-ethylphenol were inhibitory at nanomolar doses. This work is important because it shows that very low doses of cigarette smoke components significantly inhibit proper oviductal functioning raising questions regarding the safety of these compounds.

COMMENTS: POSTER PRESENTATION -- Paper N/A

ETHYL BUTYRATE
CAS: 105-54-4

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL DECANOATE
CAS: 110-38-3

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL HEXANOATE
CAS: 123-66-0
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL ISOVALERATE
CAS: 108-64-5
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL LACTATE
CAS: 97-64-3
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL LAURATE
CAS: 106-33-2
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL MYRISTATE
CAS: 124-06-1
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL OCTANOATE
CAS: 106-32-1
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL PHENYLACETATE
CAS: 101-97-3
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

2-ETHYL-3,(5 OR 6)-DIMETHYLPYRAZINE
CAS: 27043-05-6
CAS: 13925-07-0
CAS: 13360-65-1
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

5-ETHYL-3-HYDROXY-4-METHYL-2(5H)-FURANONE
CAS: 698-10-2
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

HEXYL PHENYLACETATE
CAS: 5421-17-0
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ISOAMYL ACETATE
CAS: 123-92-2
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ISOBUTYL CINNAMATE
CAS: 122-67-8
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ISOBUTYL PHENYLACETATE
CAS: 102-13-6
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ALPHA-ISOBUTYLPHENETHYL ALCOHOL (BENZYL ISOBUTYL CARBINOL) (BENZENEETHANOL, ALPHA- (2-METHYLPROPYL)-)
CAS: 7779-78-4
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ISOBUTYRIC ACID
CAS: 79-31-2
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

2-,5-, OR 6-METHOXY-3-METHYLPYRAZINE
CAS: 2847-30-5

Number of relevant papers: 1

1. Pyrazine derivatives in cigarette smoke inhibit hamster oviductal functioning

Karen Riveles, Ryan Roza , Janet Arey and Prue Talbot
Reproductive Biology and Endocrinology 2004, 2:23 doi:10.1186/1477-7827-2-23

ABSTRACT: Our past studies have shown that cigarette smoke inhibits oviductal functioning in vivo and in vitro. The goals in this study were to identify pyrazine derivatives in cigarette smoke solutions that inhibit ciliary beat frequency, oocyte pickup rate, and infundibular smooth muscle contraction in the hamster oviduct and to determine their lowest observable adverse effect levels (LOAELs) using in vitro bioassays. Methods:MS smoke solutions were fractionated using solid phase extraction cartridges and the fractions were both tested on the hamster oviduct in vitro and analyzed by gas chromatography-mass spectrometry to identify individual pyrazine derivatives. Commercial pyrazine standards were purchased, assayed for purity, and tested in dose-response studies on hamster oviducts. The LOAEL and efficacy were determined for each compound in the in vitro bioassays. Statistical significance was determined using the Student's t-Test where $p < 0.05$. Results: The LOAELs for the most inhibitory pyrazine derivatives in the ciliary beat frequency, oocyte pickup rate, and infundibular smooth muscle contraction assays were as follows: for pyrazine (1 picomolar, 10 picomolar, and 1 nanomolar); for 2-methylpyrazine (1 picomolar, 10 picomolar, and 10 picomolar); and for 2-ethylpyrazine (1 picomolar, 10 picomolar, and 1 picomolar). Six of the seven pyrazine derivatives tested (pyrazine, 2-methylpyrazine, 2-ethylpyrazine, 2-methoxy-3-methylpyrazine, 2,5-dimethylpyrazine, and 2,3,5-trimethylpyrazine) were inhibitory in picomolar or nanomolar doses in all three bioassays, while the seventh derivative, 2,6-dimethylpyrazine, had LOAELs in the nanomolar to micromolar range.

COMMENTS: An *in vitro* assay was used to study the effects of pyrazine and its derivatives in cigarette smoke on hamster oviductal functioning. Pyrazine derivatives had equal or greater effects than pyrazine and inhibited ciliary beating, oocyte pickup rate and smooth muscle contraction in hamster oviductal explants at nanomolar and picomolar doses. Oocyte pickup rate was the most sensitive parameter, and derivatives with single ethyl or methyl substitutions were among the most inhibitory. The 2-methoxy-3-methylpyrazine LOAELs for oocyte pickup rate (10^{-12} M) and muscle contraction assays (10^{-12} M) were the lowest of all pyrazines tested. The LOAEL for ciliary beat frequency (10^{-9} M) was similar to the trimethyl substritured pyrazines. The authors suggested that these data concur with results reported from in vivo hamster studies and epidemiological studies that show increased risk of ectopic pregnancies and spontaneous abortions in female smokers. The authors recommend that further toxicological testing of pyrazines be conducted.

2-METHYLHEPTANOIC ACID
CAS: 1188-02-9

Number of relevant papers: 1

1. Evaluation of certain food additives and contaminants -

Sixty-first report of the Joint FAO/WHO Expert Committee on Food Additives
WHO Technical Report Series 922, 2004 Geneva

ABSTRACT: This report represents the conclusions of a Joint FAO/WHO Expert Committee convened to evaluate the safety of various food additives, with a view to recommending acceptable daily intakes (ADIs) and to prepare specifications for the identity and purity of food additives.The first part of the report contains a general discussion of the principles governing the toxicological evaluation of food additives (including flavouring agents) and contaminants,assessments of intake, and the establishment and revision of specifications for food additives. A summary follows of the Committee's evaluations of toxicological and intake data on various specific food additives (a-amylase from Bacilluslichenformis containing a genetically engineered a-amylase gene from B. licheniformis, annatto extracts, curcumin, diacetyl and fatty acid esters of glycerol, D-tagatose, laccase from Myceliophthora thermophila expressed in Aspergillus oryzae, mixed xylanase, b-glucanase enzyme preparation produced by a strain of Humicola insolens, neotame, polyvinyl alcohol, quillaia extracts and xylanase from Thermomyces lanuginosus expressed in Fusarium venenatum), flavouring agents, a nutritional source of iron (ferrous glycinate, processed with citric acid), a disinfectant for drinking-water (sodium dichloroisocyanurate) and contaminants (cadmium and methylmercury). Annexed to the report are tables summarizing the Committee's recommendations for ADIs of the food additives, recommendations on the flavouring agents considered, and tolerable intakes of the contaminants considered, changes in the status of specifications and further information requested or desired.

COMMENTS: The abstract describes this report well.

2-METHYLPYRAZINE
CAS: 109-08-0

Number of relevant papers: 3

1. Pyrazine derivatives in cigarette smoke inhibit hamster oviductal functioning –

Karen Riveles , Ryan Roza , Janet Arey and Prue Talbot
Reproductive Biology and Endocrinology 2004, 2:23 doi:10.1186/1477-7827-2-23

ABSTRACT: Our past studies have shown that cigarette smoke inhibits oviductal functioning in vivo and in vitro. The goals in this study were to identify pyrazine derivatives in cigarette smoke solutions that inhibit ciliary beat frequency, oocyte pickup rate, and infundibular smooth muscle contraction in the hamster oviduct and to determine their lowest observable adverse effect levels (LOAELs) using in vitro bioassays.
Methods : MS smoke solutions were fractionated using solid phase extraction cartridges and the fractions were both tested on the hamster oviduct in vitro and analyzed by gas chromatography-mass spectrometry to identify individual pyrazine derivatives. Commercial pyrazine standards were purchased, assayed for purity, and tested in dose-response studies on hamster oviducts. The LOAEL and efficacy were determined for each compound in the in vitro bioassays. Statistical significance was determined using

the Student's t-Test where $p < 0.05$. Results: The LOAELs for the most inhibitory pyrazine derivatives in the ciliary beat frequency, oocyte pickup rate, and infundibular smooth muscle contraction assays were as follows: for pyrazine (1 picomolar, 10 picomolar, and 1 nanomolar); for 2-methylpyrazine (1 picomolar, 10 picomolar, and 10 picomolar); and for 2-ethylpyrazine (1 picomolar, 10 picomolar, and 1 picomolar). Six of the seven pyrazine derivatives tested (pyrazine, 2-methylpyrazine, 2-ethylpyrazine, 2-methoxy-3-methylpyrazine, 2,5-dimethylpyrazine, and 2,3,5-trimethylpyrazine) were inhibitory in picomolar or nanomolar doses in all three bioassays, while the seventh derivative, 2,6-dimethylpyrazine, had LOAELs in the nanomolar to micromolar range.
Conclusion: This work shows that very low doses of pyrazines significantly inhibit proper oviductal functioning, raising questions regarding the safety of these compounds in cigarettes and other consumer products.

COMMENTS: An *in vitro* assay was used to study the effects of to pyrazine and its derivatives in cigarette smoke on hamster oviductal functioning. Pyrazine derivatives had equal or greater effects than pyrazine and inhibited ciliary beating, oocyte pickup rate and smooth muscle contraction in hamster oviductal explants at nanomolar and picomolar doses. Oocyte pickup rate was the most sensitive parameter, and derivatives with single ethyl or methyl substitutions were among the most inhibitory. 2-methylpyrazine was one of the most potent derivatives in this study, causing effects at concentrations as low as 10^{-12} M. The authors suggested that these data concur with results reported from in vivo hamster studies and epidemiological studies that show increased risk of ectopic pregnancies and spontaneous abortions in female smokers. The authors recommend that further toxicological testing of pyrazines be conducted.

2. The influence of cigarette moisture to the chemistry of particulate phase smoke of a common commercial cigarette

Q. Zha and S.C. Moldoveanu
Beiträge zur Tabakforschung International/Contributions to Tobacco Research Volume 21(3):184-191

ABSTRACT: This study presents the results on the influence of cigarette moisture content to the chemical composition of particulate phase smoke. Seventy-five selected compounds were monitored for the comparison of particulate phase smoke of a commercial full-flavored (FF) cigarette with three different moisture contents at 7.8%, 14.5% and 20.4%, respectively. It was demonstrated that the smoke of a dry cigarette is richer in lower molecular mass compounds than a regular cigarette. On the other hand, the smoke of a moist cigarette is richer in higher molecular mass compounds than a regular cigarette. To maximize the influence of cigarette moisture to the chemical composition, a separate set of measurements were done using only the first three puffs of smoke. The accumulation of moisture in the tobacco column of a burning cigarette may influence the smoke composition, as generated during burning. The differences between dry, regular and moist cigarettes were more obvious for the first three puffs.

COMMENTS; While this is not a health effect study, the results are interesting. These investigators compared the chemical composition of cigarette smoke from cigarettes with three moisture levels (dry-8.3%, regular-11.6%, and moist- 12.9%). The first three puffs showed the greatest differences. The nicotine content and total particulate matter (TPM) were reduced with increasing moisture. The data would indicate that the dry cigarette had a higher percentage of semi-volatile compounds in TPM. The data presents additional evidence that the cigarette moisture content significantly affects the chemistry of the particulate phase of smoke. Of the 75 compounds tested the more volatile compounds were more affected than the less volatile compounds. Compared to the first three puffs, the particulate phase of smoke from the entire cigarette was less sensitive to the moisture content.

3. Growth and Angiogenesis Are Inhibited in Vivo in Developing Tissues by Pyrazine and Its Derivatives –

Goar Melkonian, Holly Lautenschlager, Melinda Wu, Yuhuan Wang, Cathy Tong, Karen Riveles, P. Talbot.
Toxicological Sciences Volume 75, Number 2 Pp. 393-401

ABSTRACT: Sidestream cigarette smoke solution was previously screened to identify the groups of chemicals in smoke that inhibit growth and angiogenesis in the chick chorioallantoic membrane (CAM). Pyrazine and several pyrazine derivatives were identified as a major chemical group in this screen. In the current study, purified pyrazine and six pyrazine derivatives identified in the screen were tested in dose response experiments to measure their effects on CAM growth, embryo growth, and angiogenesis. Chemicals or control medium were placed on CAMs in ovo on day 5 of development, and results were evaluated on day 6. Of the chemicals tested, pyrazine was the most potent and inhibited both CAM and embryo growth at picomolar doses. 2-ethylpyrazine and 2,3, dimethylpyrazine were inhibitory at nanomolar doses. Inhibition of growth by pyrazine was correlated with inhibition of DNA synthesis. The pattern of blood vessel development in CAMs was disturbed by micromolar doses of pyrazine and 2,3,-dimethylpyrazine. Migration of mesodermal blood vessels to the ectoderm of CAMs and their subsequent differentiation into the capillary plexus was impaired by nanomolar doses of pyrazine. In summary, these data show that pyrazine and some of its derivatives inhibit growth and certain process important in angiogenesis at very low doses. Since pyrazine and some of its derivatives are considered safe food additives, further toxicological testing of pyrazine, in particular on developing tissues, should be done to fully evaluate its safety as a consumer product additive.

COMMENTS: These authors previously presented data that both mainstream and sidestream smoke inhibit growth and angiogenesis in chick chorioallantoic membrane (CAM). The CAM is important to the chick since it serves as the respiratory organ for gaseous exchange until hatching. These studies are an extension of their earlier work in hope to identify the compound responsible for this effect. The data show that pyrazine in sidesteam smoke can inhibit CAM, embryo growth and impair angiogenesis in nano and picomolar doses. Of the pyrazines tested, 2-methylpyrazine significantly inhibited CAM

growth at $5X10^{-5}$ M but did not significantly affect embryo growth at any dose tested. The authors state that the implications of this data to human reproduction are not known.

GAMMA-OCTALACTONE
CAS: 104-50-7
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

2,3-PENTANEDIONE
CAS: 600-14-6
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

2-PHENETHYL ACETATE
CAS: 103-45-7
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

PHENYLACETALDEHYDE
CAS: 122-78-1
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

SODIUM BICARBONATE
CAS: 144-55-8
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

SUCROSE OCTAACETATE
CAS: 126-14-7

Number of relevant papers: 1

1. The Contribution of Taste Bud Populations to Bitter Avoidance in Mouse Strains Differentially Sensitive to Sucrose Octa-acetate and Quinine -

St John SJ, Boughter JD Jr.
Chem Senses. 2004 Nov;29(9):775-87.

ABSTRACT: Mice of the SWR/J (SW) strain avoid orally delivered sucrose octa-acetate (SOA), whereas the mice of the C3HeB/FeJ (C3) strain are insensitive to SOA. Mice of both strains and of a congenic strain (C3.SW) that shares more than 99% of the C3 genome, were tested in a taste-salient brief-access taste test for responses to SOA and quinine hydrochloride, before and after transection of the glossopharyngeal or chorda tympani nerve, or sham surgery. Prior to surgery, congenic SOA tasters (C3.SW(T)) were

phenotypically identical to the SW strain in avoidance of SOA, but showed a greater reduction in sensitivity after nerve transection. For quinine avoidance, which is thought to be a polygenic trait, SW mice showed the greatest sensitivity to quinine, C3 the least and C3.SW(T) mice were different from both parental strains, showing intermediate sensitivity. Nerve transections had only a moderate effect on quinine sensitivity, suggesting that both anterior and posterior taste bud fields contribute to behavioral quinine avoidance. These findings are discussed with regard to the distribution in the oral cavity of putative taste receptors for quinine and SOA and the peripheral organization of bitter taste.

COMMENTS: This paper has to do with taste receptors. Although it is not directly health-related, it may be of interest.

2,3,5,6-TETRAMETHYLPYRAZINE
CAS: 1124-11-4
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

TRIETHYL CITRATE
CAS: 77-93-0
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

4-(2,6,6-TRIMETHYLCYCLOHEX-1-ENYL)BUT-2-EN-4-ONE (BETA-DAMASCONE)
CAS: 23726-91-2
CAS: 35044-68-9
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

CATEGORY: MAJOR INGREDIENTS

GLYCEROL
CAS: 56-81-5

Number of relevant papers: 1

1. Glycerol transfer in cigarette mainstream smoke

C.Liu.
Beitrage Tabakforschung Int., 21 (2004) No. 2, pp.111-116.

ABSTRACT: Experiments have been conducted to examine the effect of different levels of blend cigarette at 36 for a 11.4 blend glycerol. For cigarettes with different designs the glycerol in NFDPM may also depend on the glycerol loading per unit rod length. The tobacco rod filtration did not change significantly within the glycerol range investigated and hence plays a relatively minor role. Significant glycerol condensation ahead of the burning coal after a puff was measured. This condensation may have implications on glycerol levels in the sidestream smoke during inter-puff smouldering.

COMMENTS: While this is not a health effect study, it does present data on the transfer of glycerol into MSS when added at higher levels than normally used as a humectant. The author's conclusion was that 1. mainstream glycerol yield increased with the blend glycerol levels, 2. the tobacco rod filtration was not significantly altered by glycerol levels, and 3. significant glycerol condensation was found ahead of the burning coal after a puff. Unfortunately, the levels of glycerol in the sidestream smoke, butt and filter were not measured.

CARBON
CAS: 7440-44-0

Number of relevant papers: 4

GENERAL COMMENTS ON CARBON AND GRAPHITE

There are numerous inhalation studies on the health effects associated with exposure to a variety of carbonaceous materials including activated carbon, graphite, coal dust, lamp black, soot, and diesel emissions. Some of these materials appear to cause tumors in rats when inhaled chronically at high concentrations. Such reports may be of interest since carbon-based particles can have absorbed onto them a variety of organic compounds including polycyclic aromatic hydrocarbons, nitroaromatic compounds, and heterocyclic compounds. The presence of similar organic substances in smoke may also be absorbed onto any carbon particles which theoretically could act synergistically or additively producing a greater response as compared to single components. Such effects may be involved in tumor development and DNA damage through both the chemical and particulate-mediated cytotoxicity responses. Particle size has a significant role in the toxicity of inhaled particles. For example, studies would indicate that ultrafine particles (<0.1 μm diameter) produce significantly greater inflammatory response than do fine particles per given mass. Such inhaled particles can lead to production of a number of mediators such as reactive oxygen and nitrogen species, cytokines, growth factors and other substances that might mediate tissue injury and contribute to the pathogenesis of pulmonary disease. Studies also suggest an excess risk of esophageal cancer, particularly squamous cell carcinomas, with exposure to carbon black combined with acid aerosols. However, the level of exposure in many of these studies was several orders of magnitude higher than one would expect from cigarette smoke inhalation.

It is important to differentiate between the types of carbon-based particles. Carbon black, for example, is manufactured under controlled conditions, while the soot-types of carbon contain numerous unwanted byproducts from the combustion of carbon-based materials. Often the terms carbon black and soot are used interchangeably. However, they are physically and chemically distinct. Soots have a much greater percentage of ash and more organic compounds can be extracted from particle surfaces.

Increases in human cancers have been attributed to high exposure to carbon black dust during working conditions and it has been classified as a possible lung and bladder carcinogen by IRAC. Carbon black is mutagenic in the Ames assay. Inhaled carbon black has also been shown to be carcinogenic in rat bioassays. For example, exposure for 24 mo to carbon black 16 hr/day, for 5 days/wk at a concentration of 2.5 or 6.5 mg/m^3 produced malignant and benign lung tumors. In such cases, clearance was impaired and particles accumulated progressively. There is evidence that lung overloading is a requisite for induction of lung tumors in this animal model. A similar range of tumor phenotypes has been reported in the lungs of rats exposed to high concentrations of diesel exhaust and coal dust. Intratracheal instillation of carbon black resulted in a dose-response neutrophil inflammation. Bronchoalveolar lavage cell population was associated with increased mutation rates in alveolar type II epithelial cells. Subchronic inhalation studies in rats did not show increases in mutation frequency at a concentration of 1.1 mg/m^3, and lung clearance was not impaired at that level of exposure.

1. Inhaled particles and lung cancer, part B: Paradigms and risk assessment -

Borm PJ, Schins RP, Albrecht C.
Int J Cancer. 2004 May 20;110(1):3-14.

ABSTRACT: Poorly soluble particles of low toxicity (PSP), such as CB, TiO(2) and coal mine dust, have been demonstrated to cause lung cancer in rodents, being most pronounced in rats. Adequate epidemiologic studies do not clearly indicate increased lung cancer rates in humans exposed to such particles. This has caused controversial positions in regulatory decisions on PSP on different levels. The present review discusses the current paradigms in rodent particle carcinogenicity, i.e., (i) role of particle overload and of persistent inflammation and (ii) fibrosis as an intermediate step in particle-induced lung cancer with regard to human risk assessment. Fibrosis, which is usually considered a precursor of lung cancer in humans, was not related to lung tumors in an animal study using 6 different particles, each at 3 dosages. Lung tumors after both inhalation and intratracheal instillation of PSP are related to particle surface dose, which forwards hazard assessment at surface-based nonoverload concentrations and a standard setting using surface as an exposure metric. The scarce data available on humans do not support the overload concept but suggest a role for persistent lung inflammation. Differences in antioxidant protection between different rodent species correlate with susceptibility to PSP-induced carcinogenicity and support the need for detailed studies on antioxidant response in humans. Apart from such bridging studies, further focus is also needed on surface chemistry and modifications in relation to their adverse biologic effects.

COMMENTS: This manuscript reviews the possible mechanism of action associated with particle-induced lung carcinogenesis. These authors attempt to explain why a number of poorly soluble particles (PSP), such as carbon black and graphite, has been shown to be carcinogenic in the rat and may or may not be carcinogenic in humans. The extrapolation of these rodent studies to humans is difficult because of lack of knowledge regarding antioxidant response, the significance of inflammation in the process of genotoxicity and proliferations in the human lung. The authors state that all inhaled particles are likely to induce tumors in the rat model if the particles are inhaled or instilled at sufficiently high doses and highly durable. While carbon has been identified as a possible carcinogen by IRAC, based on these rodent studies, these authors state that this action may be premature and needs further consideration.

2. Pulmonary and systemic effects of short-term inhalation exposure to ultrafine carbon black particles -

Gilmour PS, Ziesenis A, Morrison ER, Vickers MA, Drost EM, Ford I, Karg E, Mossa C, Schroeppel A, Ferron GA, Heyder J, Greaves M, MacNee W, Donaldson K.
Toxicol Appl Pharmacol. 2004 Feb 15;195(1):35-44.

ABSTRACT: While environmental particles are associated with mortality and morbidity related to pulmonary and cardiovascular (CV) disease, the mechanisms involved in CV health effects are not known. Changes in systemic clotting factors have been associated with pulmonary inflammation. We hypothesized that inhaled ultrafine particles result in an inflammatory response which may stimulate systemic clotting factor release. Adult male Wistar rats were exposed to either fine or ultrafine carbon black (CB) for 7 h. The attained total suspended particle concentrations were 1.66 mg/m(3) for ultrafine CB and 1.40 mg/m(3) for fine CB. Particle concentration of ultrafine particles was more than 10 times greater than that of fine particles and the count median aerodynamic diameter averaged 114 nm for the ultrafine and 268 nm for the fine carbon particles. Data were collected immediately, 16 and 48 h following exposure. Only ultrafine CB caused an increase in total bronchoalveolar lavage (BAL) leukocytes, whereas both fine (2-fold) and ultrafine (4-fold) carbon particles caused an increase in BAL neutrophils at 16 h postexposure. Exposure to the ultrafine, but not fine, carbon was also associated with significant increases in the total numbers of blood leukocytes. Plasma fibrinogen, factor VII and von Willebrand factor (vWF) were unaffected by particle treatments as was plasma Trolox equivalent antioxidant status (TEAC). Macrophage inflammatory protein-2 mRNA was significantly increased in BAL cells 48 h following exposure to ultrafine CB. The data show that there is a small but consistent significant proinflammatory effect of this exposure to ultrafine particles that is greater than the effect of the same exposure to fine CB.

COMMENTS: In this study, rats were exposed by inhalation to approximately 1.5 mg/m^3 fine and utrafine carbon black (CB) particles. Following a single 7-h exposure, the bronchoalveolar lavage (BAL) inflammatory profile was assessed at 0, 16, and 48 hours post-exposure. A total deposition of 3.9 µg particle mass in the deep lung was estimated.

The results indicate that particle size is an important determinant of pulmonary responses to CB, since exposure to ultrafine CB particles was associated with effects not seen following fine CB particle exposure. An increase in BAL cells in rats exposed to ultrafine CB was observed as well as an increase in the number of neutrophils (PMNs) in the BAL fluid and an increase in blood leukocytes. No effects on blood coagulation factors or plasma antioxidant capacity were observed. These findings are consistent with previous studies of acute human exposure to concentrated ambient particles, with the exception that an increase in blood fibrinogen was observed in humans. The authors note that difference in findings between the two studies may be due to species differences or the more heterogeneous nature of ambient particles used in the human studies.

3. Immunological biomarkers in salt miners exposed to salt dust, diesel exhaust and nitrogen oxides -

Backe E, Lotz G, Tittelbach U, Plitzko S, Gierke E, Schneider WD.
Int Arch Occup Environ Health. 2004 Jun;77(5):319-27. Epub 2004 Jun 12

ABSTRACT: Air pollutants can affect lung function and also the immune system. In a study about lung function of salt miners in relation to the complex exposure in a salt mine, we also analysed selected immunological parameters and inflammation markers in the blood of miners. Effect of salt dust, diesel exhaust, nitrogen oxides (NOx) and smoking on the biomarkers was analysed. METHODS: Blood was drawn from 286 salt miners, and the soluble intercellular adhesion molecule-1 (s-ICAM), monocyte chemotactic protein (MCP-1) and clara cell protein (CC16) were analysed by an immunoassay, blood profile was done and lymphocyte subpopulations (CD3, CD3/CD4, CD3/CD8, CD19, NK-cells, CD3/HLA-DR) were determined by flow cytometry. Salt dust was measured by two-step gravimetry (personal sampling). Diesel exhaust was measured as elemental carbon concentration by coulometry. NOx were determined by an electrochemical cell method. Differences between non-smokers, former smokers and active smokers were analysed by analysis of variance. Linear regression analysis to describe exposure-response relationships was done with regard to confounding factors [smoking, inflammatory diseases, time of blood drawing, respiratory infection and body-mass index (BMI)]. RESULTS: Significant differences between non-smokers and active smokers were found for most of the leukocyte types (e.g. granulocytes $P = 0.000$, lymphocytes $P = 0.002$, T-cells $P = 0.033$) and for some soluble parameters (ICAM $P = 0.000$, IgM $P = 0.007$, IgE $P = 0.035$). Increasing numbers of total lymphocytes, T-cells and HLA-DR positive T-cells in relation to exposure were found by linear regression analysis (e.g. for inhalable dust:total lymphocytes $P = 0.011$, T-cells $P = 0.061$, HLA-DR positive T-cells $P = 0.007$). CONCLUSION. Comparison of immunological markers in non-smokers and active smokers confirms leukocytosis and inflammation following tobacco consumption. The combined exposure of salt dust, diesel exhaust and NOx seems to influence the immune system. Together, the results suggest that the analysis of leukocytes and their subsets can complete other investigations (lung function, questionnaire) to monitor exposure-response relationships in occupational studies investigating the effect of inhaled substances. Longitudinal studies will be necessary to

determine the predictive value of the immunological changes. Copyright 2004 Springer-Verlag

COMMENTS: Immunological parameters and inflammation markers were assessed in salt mine workers exposed to complex mixtures of salt dust, nitrogen oxides and diesel exhaust. These same markers were also evaluated in relation to tobacco smoke exposure. Exposure –dependent increases in lymphocytes, T-cells and activated T-cells indicated an effect on the immune system, however, it was not possible to distinguish between the contributions of the different exposure types. The effect of exposure to these mixtures was confounded by smoking and body-mass index which contributed to alterations in the number of immunocompetent cells. Differences between smokers and nonsmokers included increases in immune cells and some soluble markers in blood. Lymphocytes and T-cells were positively correlated with the number of cigarettes smoked per day. Extrapolation of the findings of this study to the health effects of carbon as an ingredient in cigarettes is difficult.

4. Ultrafine particle deposition in subjects with asthma -

David C. Chalupa, Paul E. Morrow, Günter Oberdörster, Mark J. Utell, and Mark W. Frampton
Environmental Health Perspectives Volume 112, Number 8, June 2004

Abstract: Ambient air particles in the ultrafine size range (diameter < 100 nm) may contribute to the health effects of particulate matter. However, there are few data on ultrafine particle deposition during spontaneous breathing, and none in people with asthma. Sixteen subjects with mild to moderate asthma were exposed for 2 hr, by mouthpiece, to ultrafine carbon particles with a count median diameter (CMD) of 23 nm and a geometric standard deviation of 1.6. Deposition was measured during spontaneous breathing at rest (minute ventilation, 13.3 ± 2.0 L/min) and exercise (minute ventilation, 41.9 ± 9.0 L/min). The mean ± SD fractional deposition was 0.76 ± 0.05 by particle number and 0.69 ± 0.07 by particle mass concentration. The number deposition fraction increased as particle size decreased, reaching 0.84 ± 0.03 for the smallest particles (midpoint CMD = 8.7 nm). No differences between sexes were observed. The deposition fraction increased during exercise to 0.86 ± 0.04 and 0.79 ± 0.05 by particle number and mass concentration, respectively, and reached 0.93 ± 0.02 for the smallest particles. Experimental deposition data exceeded model predictions during exercise. The deposition at rest was greater in these subjects with asthma than in previously studied healthy subjects (0.76 ± 0.05 vs. 0.65 ± 0.10, $p < 0.001$). The efficient respiratory deposition of ultrafine particles increases further in subjects with asthma. Key words: air pollution, asthma, deposition, dosimetry, inhalation, ultrafine particles. Environ Health Perspect 112:879-882 (2004). doi:10.1289/ehp.6851 available via http://dx.doi.org/ [Online 2 March 2004]

COMMENTS: This study focused on ultrafine particle (UFP) deposition in individuals with asthma. The hypothesis was if lung dose of UFP are higher for individuals with asthma, than the health risk might also increase. Previous studies have shown that

individuals with chronic obstructive pulmonary disease have enhanced deposition of both fine and ultrafines. These results indicated that when both increased deposition fraction and minute ventilation were considered, the total number of carbon particles retained in the lung was 74% greater in subjects with asthma than healthy subjects which may make them more susceptible to respiratory disease.

GRAPHITE
CAS: 7782-42-5

Number of relevant papers: 2

1. Translocation of inhaled ultrafine particles to the brain -

Oberdorster G, Sharp Z, Atudorei V, Elder A, Gelein R, Kreyling W, Cox C.
Inhal Toxicol. 2004 Jun;16(6-7):437-45.

ABSTRACT: Ultrafine particles (UFP, particles <100 nm) are ubiquitous in ambient urban and indoor air from multiple sources and may contribute to adverse respiratory and cardiovascular effects of PM (Particulate Matter). Depending on their particle size, inhaled UFP are efficiently deposited in nasal, tracheobronchial and alveolar regions due to diffusion. Our previous rat studies have shown that UFP can translocate to interstitial sites in the respiratory tract as well as to extrapulmonary organs such as liver within 4-24 hrs. post-exposure. There were also indications that the olfactory bulb of the brain was targeted. Our objective in this follow-up study, therefore, was to determine whether translocation of inhaled ultrafine solid particles to regions of the brain takes place, hypothesizing that UFP depositing on the olfactory mucosa of the nasal region will translocate along the olfactory nerve into the olfactory bulb. This should result in significant increases in that region on the days following the exposure as opposed to other areas of the CNS. We generated ultrafine elemental 13C particles (CMD = 36 nm; GSD = 1.66) from 13C graphite rods by electric spark discharge in an argon atmosphere at a concentration of 160 μg/m3. Rats were exposed for 6 hrs. and lungs, cerebrum, cerebellum and olfactory bulbs were removed 1,3,5 and 7 days after exposure. 13C concentrations were determined by isotope ratio mass spectroscopy and compared to background 13C levels of sham-exposed controls (day 0). The background corrected pulmonary 13C added as ultrafine 13C particles on day 1 post-exposure was 1.34 μg/lung. Lung 13C concentration decreased from 1.39 μg/g (day 1) to 0.59 μg/g by 7 days post-exposure. There was a significant and persistent increase in added 13C in the olfactory bulb of 0.35 μg/g on day 1 which increased to 0.43 μg/g by day 7. Day 1 13C concentrations of cerebrum and cerebellum were also significantly increased but the increase was inconsistent, significant only on one additional day of the post-exposure period, possibly reflecting translocation across the blood-brain barrier in certain brain regions. The increases in olfactory bulbs are consistent with earlier studies in non-human primates and rodents which demonstrated that intranasally-instilled solid UFP translocate along axons of the olfactory nerve into the CNS. We conclude from our study that the CNS can be targeted by airborne solid ultrafine particles and that the most likely

mechanism is from deposits on the olfactory mucosa of the nasopharyngeal region of the respiratory tract and subsequent translocation via the olfactory nerve. Depending on particle size, >50% of inhaled UFP can be depositing in the nasopharyngeal region during nasal breathing. Preliminary estimates from the present results show that ~20% of the UFP deposited on the olfactory mucosa of the rat can be translocated to the olfactory bulb. Such neuronal translocation constitutes an additional not generally recognized clearance pathway for inhaled solid UFP, whose significance for humans, however, still needs to be established. It could provide a portal of entry into the CNS for solid UFP, circumventing the tight blood-brain barrier. Whether this translocation of inhaled UFP can cause CNS effects needs to be determined in future studies.

COMMENTS: These authors report that they found significant and continuous increases of ultrafine particles in the olfactory bulb throughout a 7 day inhalation exposure. These results suggest that inhaled ultrafine carbon particles are translocated to the CNS. This provides evidence of a direct portal of entry for ultrafines into the CNS. Such evidence could indicate potential long term effects and accumulation of such particles to other regions of the CNS.

2. Inhaled particles and lung cancer, part B: Paradigms and risk assessment -

Borm PJ, Schins RP, Albrecht C.
Int J Cancer. 2004 May 20;110(1):3-14.

ABSTRACT: Poorly soluble particles of low toxicity (PSP), such as CB, TiO(2) and coal mine dust, have been demonstrated to cause lung cancer in rodents, being most pronounced in rats. Adequate epidemiologic studies do not clearly indicate increased lung cancer rates in humans exposed to such particles. This has caused controversial positions in regulatory decisions on PSP on different levels. The present review discusses the current paradigms in rodent particle carcinogenicity, i.e., (i) role of particle overload and of persistent inflammation and (ii) fibrosis as an intermediate step in particle-induced lung cancer with regard to human risk assessment. Fibrosis, which is usually considered a precursor of lung cancer in humans, was not related to lung tumors in an animal study using 6 different particles, each at 3 dosages. Lung tumors after both inhalation and intratracheal instillation of PSP are related to particle surface dose, which forwards hazard assessment at surface-based nonoverload concentrations and a standard setting using surface as an exposure metric. The scarce data available on humans do not support the overload concept but suggest a role for persistent lung inflammation. Differences in antioxidant protection between different rodent species correlate with susceptibility to PSP-induced carcinogenicity and support the need for detailed studies on antioxidant response in humans. Apart from such bridging studies, further focus is also needed on surface chemistry and modifications in relation to their adverse biologic effects.

COMMENTS: See General Comments for this paper under the Carbon Ingredient listing.

INVERT SUGAR
CAS: 8013-17-0
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

MAPLE SYRUP
CAS: 8029-81-0
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

HIGH FRUCTOSE CORN SYRUP
8029-43-4
977042-84-4
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

CORN SYRUP
8029-43-4
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

CELLULOSE AND CELLULOSE FIBER
65996-61-4
09004-34-6
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

SUCROSE
CAS: 57-50-1

Number of relevant papers: 3

1. Sucrose and IQ induced mutations in rat colon by independent mechanism

Hansen M, Hald MT, Autrup H, Vogel U, Bornholdt J, Moller P, Molck AM, Lindecrona R, Poulsen HE, Wallin H, Loft S, Dragsted LO.
Mutat Res. 2004 Oct 4;554(1-2):279-86.

ABSTRACT: Sucrose-rich diets have repeatedly been observed to have co-carcinogenic actions in colon and liver of rats and to increase the number of 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) induced aberrant crypt foci in rat colon. To investigate a possible interaction between sucrose and IQ on the genotoxicity in rat liver and colon, we gave Big Blue ratsTM a diet containing sucrose (0%, 3.45% or 13.4% w/w) and/or IQ (70 ppm) for a period of 3 weeks. Sucrose and IQ increased the mutation frequency in the colon. The effect of combined treatments with IQ and sucrose on the mutation frequencies was additive indicating that sucrose and IQ act independently. This

was supported by the mutation spectra where sucrose expands the backgroundmutations in the colon, whereas IQ, in other studies, more specifically has induced G:C → T:A transversions. In the liver IQ increased the mutation frequency, whereas addition of sucrose reduced the effect of IQ in a dose-dependent manner. The level of bulky DNA adducts in liver and colon was increased in animals exposed to either sucrose or IQ. In animals exposed to IQ, addition of sucrose had marginal effects on the level of bulky DNA adducts. Markers of oxidative damage and DNA repair were generally unaffected by the treatments. In conclusion, sucrose and IQ in the diet induced mutations in the colon by independent mechanisms, whereas an interaction was observed in liver leading to a decrease in mutations by the combined treatment.

COMMENTS: The interaction between high doses (3.4 and 13.45%) of sucrose and 2-amino-3-methylimidazo [4,5-*f*]quinoline (IQ) which is a strong hepatic carcinogen in non-human primates) were assessed in rats using 3-week dietary exposures. The authors state that this study confirms previous reports of the mutagenic effects of sucrose in the rat colon. In the liver, they report a decrease in mutation frequencies with increased levels of sucrose however, the level of DNA adducts was increased by sucrose in both the colon and liver, possibly indicating that other factors may be influencing the mutagenic effects.

2. Assessment of the performance of the Ames II assay: a collaborative study with 19 coded compounds –

Fluckiger-Isler S, Baumeister M, Braun K, Gervais V, Hasler-Nguyen N, Reimann R, Van Gompel J, Wunderlich HG, Engelhardt G.
Mutat Res. 2004 Mar 14;558(1-2):181-97.

ABSTRACT: Nineteen coded chemicals were tested in an international collaborative study for their mutagenic activity. The assay system employedwas the Ames IIMutagenicity Assay, using the tester strains TA98 and TAMix (TA7001–7006). The test compounds were selected from a published study with a large data set from the standard Ames plate-incorporation test. The following test compounds including matched pairs were investigated: cyclophoshamide, 2-naphthylamine, benzo(a)pyrene, pyrene, 2-acetylaminofluorene, 4,4_-methylene-bis(2-chloroaniline), 9,10-dimethylanthracene, anthracene, 4-nitroquinoline-N-oxide, diphenylnitrosamine, urethane, isopropyl-N(3-chlorophenyl)carbamate, benzidine, 3,3_-5,5_-tetramethylbenzidine, azoxybenzene, 3-aminotriazole, diethylstilbestrol, sucrose and methionine. The results of both assay systems were compared, and the inter-laboratory consistency of the Ames II test was assessed. Of the eight mutagens selected, six were correctly identified with the Ames II assay by all laboratories, one compound was judged positive by five of six investigators and one by four of six laboratories. All seven non-mutagenic samples were consistently negative in the Ames II assay. Of the four chemicals that gave inconsistent results in the traditional Ames test, three were uniformly classified as either positive or negative in the present study, whereas one compound gave equivocal results. A comparison of the test outcome of the different investigators resulted in an inter-laboratory consistency of 89.5%. Owing to the high concordance between the two test systems, and the low inter-

laboratory variability in the Ames II assay results, the Ames II is an effective screening alternative to the standard Ames test, requiring less test material and labor.

COMMENTS: While there are studies reporting mutagenic effects of sucrose, this study examined 19 coded compounds and came to the conclusion that sucrose was consistently negative in the Ames II assay. The Ames II assay is a liquid microtiter modification of the traditional Ames test and is considered to be a suitable alterative to the standard type Ames plate method.

3. Sucrose consumption enhances the analgesic effects of cigarette smoking in male and female smokers

Kanarek RB, Carrington C.
Psychopharmacology (Berl). 2004 Apr;173(1-2):57-63. Epub 2004 Jan 14.

ABSTRACT: Abstract Rationale: Nicotine has analgesic actions in experimental animals and humans. Moreover, the analgesic properties of nicotine in experimental animals are increased by intake of sweet-tasting nutritive fluids. It is important to determine if the effects of diet on nicotineinduced analgesia are limited to experimental animals, or if these effects can be translated from the laboratory to clinical research situations. Objective: This study investigated whether intake of a sweet-tasting sucrose solution would enhance the pain relieving actions of nicotine, administered in the form of cigarette smoking, in male and female college-aged students. The effects of smoking and sucrose intake on mood were also examined. Method: Using the cold pressor test, pain thresholds and pain tolerance were determined in 24 male and 25 female
smokers. Each participant was tested 4 times. On 2 of the test days, participants drank a sucrose-containing beverage, and on 2 of the days, drank water. Twenty-five minutes later, participants either smoked a cigarette or did not smoke. Participants were tested 5 min later for their responses on the cold pressor test. To determine if mood was altered by smoking or sucrose intake, the Profile of Mood Scale was administered immediately preceding and following experimental manipulations. Results: Cold threshold and cold tolerance were greater when participants were allowed to smoke than when they were not allowed to smoke. While men and women responded in a similar manner to the experimental manipulations, men displayed significantly greater cold threshold and cold tolerance than women. Sucrose consumption augmented the effects of smoking on cold threshold, but not on cold tolerance. Men reported feeling significantly more vigorous and less angry, and women reported feeling significantly less tense after they had smoked than when they had not smoked. Sucrose consumption did not alter self reports of mood in either men or women. Conclusion: These findings suggest that sucrose augments the analgesic properties of nicotine in humans, as well as in experimental animals, and suggest that diet could serve as an adjunct in the control of pain.

COMMENTS: This study was designed to investigate the interactions between sucrose intake and smoking on pain sensitivity and mood in humans. Cigarette smoking led to increases in pain threshold and tolerance. Sucrose intake (28.5 g, achieved by drinking a sucrose-containing beverage) increased pain threshold when combined with smoking, but

not alone. Sucrose intake also did not affect self-reported mood. The authors speculate that both sucrose and nicotine may alter central cholinergic neurons. The relevance of this study to sucrose as an ingredient in cigarettes is minor because of the differences in sucrose exposure concentration and route of exposure.

PROPYLENE GLYCOL
57-55-6

Number of relevant papers: 2

1. NTP-CERHR Expert Panel report on the reproductive and developmental toxicity of propylene glycol -

Center for the Evaluation of Risks to Human Reproduction
Reproductive Toxicology Volume 18, Issue 4 , June 2004, Pages 533-579

Abstract: The National Toxicology Program (NTP) and the National Institute of Environmental Health Sciences (NIEHS) established the NTP Center for the Evaluation of Risks to Human Reproduction (CERHR) in June 1998. The purpose of the Center is to provide timely, unbiased, scientifically sound evaluations of human and experimental evidence for adverse effects on reproduction, including development, caused by agents to which humans may be exposed. Propylene glycol was selected for evaluation by the CERHR based on its high production and widespread public exposure due to its use as an antifreeze and de-icing agent, as well as its use in paints, coatings, foods, drugs, and cosmetics. This evaluation results from the efforts of a nine-member panel of government and non-government scientists that culminated in a public expert panel meeting held February 11–13, 2003. This report has been reviewed by CERHR staff scientists and by members of the Ethylene Glycol/Propylene Glycol Expert Panel. Copies have been provided to the CERHR Core Committee, which is made up of representatives of NTP-participating agencies. This report is a product of the expert panel and is intended to (1) interpret the strength of scientific evidence that propylene glycol is a reproductive or developmental toxicant based on data from in vitro, animal, or human studies, (2) assess the extent of human exposures to include exposures of the general public, occupational groups, and other sub-populations, (3) provide objective and scientifically thorough assessments of the scientific evidence that adverse reproductive/developmental health effects may be associated with such exposures, and (4) identify knowledge gaps to help establish research and testing priorities to reduce uncertainties and increase confidence in future assessments of risk. The Expert Panel Report on Propylene Glycol will be a central part of the subsequent NTP CERHR Monograph. The monograph will include the NTP CERHR Brief, the expert panel report, and all public comments on the expert panel report. The NTP CERHR Monograph will be made publicly available and transmitted to appropriate health and regulatory agencies.

COMMENTS: This paper provides a thorough review of the use, exposure, metabolism and toxicity of propylene glycol. The panel estimates 25 million pounds (2.9% of the

total consumption) of propylene glycol was used as tobacco humectant in 1999. American Industrial Hygiene Association Workplace Environmental Exposure Level guide of 50 ppm total exposure and 10mg/m^3 inhalation aerosol exposure have been determined. Propylene glycol has a short half life and very low systemic toxicity, is not mutagenic, nor developmentally toxic. Although human inhalation exposures were considered within this review (in situations such as actors exposed to theatrical fog), studies have not included propylene glycol as an ingredient in cigarettes and data available on inhalation exposure in animals are inconclusive. The panel concluded "the current estimated exposures to propylene glycol are of negligible concern for reproductive or developmental toxicity in humans." Potentially sensitive subpopulations include patients with impaired liver or kidney function.

2. Subchronic inhalation studies of complex fragrance mixtures in rats and hamsters

Fukayama MY, Easterday OD, Serafino PA, Renskers KJ, North-Root H, Schrankel KR.
Toxicol Lett. 1999 Dec 20;111(1-2):175-87.

ABSTRACT: Users of consumer products are invariably and intentionally exposed to complex mixtures in such products. With finished fragrance products, these mixtures may represent 100 or more fragrance raw materials (FRMs). The objective of the described studies was to evaluate the safety of finished fragrance products via the inhalation route. In total, the finished products contained approximately 100 FRMs at concentrations of 1% or greater. Major FRMs evaluated included benzyl acetate, coumarin, hydroxycitronellal, musk ketone, 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethyl-cyclopenta-gamma-2-be nzopyran (HHCB) and phenyl ethyl alcohol. Groups of rats or hamsters were exposed by inhalation (whole body) to the mixtures at 5, 9 or 50 mg/m3 for 4 h per day, 5 days per week for 6 or 13 weeks. For each of the fragrance products, the doses used generally represented a ten- to 100-fold exaggeration of levels expected to be achieved during typical use by consumers. With one exception, the fragrances were aerosolized prior to introduction into the inhalation chamber. The exception product was formulated with a propellant, packaged in a pressurized container and expelled with an automated actuator. In all studies, chamber concentrations of fragrance were monitored. Particle sizes ranged from 0.5 to 7.5 microm, depending on the study. Subchronic exposure to all fragrance mixtures resulted in no toxicologically significant effects on animal survival, behavior, body weights or weight gains, organ weights, or in hematology, clinical chemistry, or urinalysis parameters. No gross pathological or histopathological findings related to test material exposures were observed. These studies support the conclusions that the fragrance mixtures would not pose a hazard to product users based on repeated and exaggerated inhalation exposures of animals.

COMMENTS: This paper presents results from subchronic inhalation studies of fragrance mixtures in rats and hamsters. Eight fragrance products were tested, which include approximately 200 components, including propylene glycol (13 – 52 μg/m^3). No toxicological effects were identified from exposure to the fragrance mixtures at levels 10- to 100 fold greater than expected human exposure conditions for 6 or 13 weeks. The

results are consistent with those earlier studies of Gaworski 1998 that exposed rats to mixtures of flavor ingredients in a subchronic smoke inhalation study.

BROWN SUGAR
CAS: 57-50-1
SEE MAJOR INGREDIENTS

HONEY
CAS: 8028-66-8
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

MENTHOL AND L-MENTHOL
CAS: 89-78-1

Number of relevant papers: 6

1. On the biological properties of fragrance compounds and essential oils - UBER BIOLOGISCHE WIRKUNGEN VON DUFTSTOFFEN UND ATHERISCHEN OLEN -

Buchbauer G.
Wien Med Wochenschr. 2004 Nov;154(21-22):539-47.

ABSTRACT: In the present review the physiological and/or pharmacological properties of essential oils and of single fragrance compounds are discussed. Essential oils are known and have been used since ancient times as natural medicines. As natural products essential oils are dependent on climate and their composition varies according to conditions of soil, to solar irradiation, to harvest time, to production methods, to storage conditions and similar facts which are discussed in chapter 2 of this review. The next chapters deal with the therapeutic use of essential oils in treating diseases, disorders or ailments of the nervous system, against cancer and as penetration enhancers. For space-saving reasons, however, the manifold antimicrobial and antifungal properties of these natural products have been left out. In the last chapter, the pros and cons in the use of essential oils in therapy are also discussed.

COMMENTS: This article is in German and was not translated.

2. Mentholated cigarette smoking inhibits nicotine metabolism

Neal L. Benowitz, Brenda Herrera, and Peyton Jacob, III
Journal of Pharmacology And Experimental Therapeutics 310:1208-1215, 2004

ABSTRACT: Smoking mentholated cigarettes has been suggested to convey a greater cancer risk compared with smoking nonmentholated cigarettes. Two of the possible mechanisms by which mentholated cigarette smoking could increase risk are by increasing systemic exposure to tobacco smoke toxins and by affecting the metabolism of nicotine or tobacco smoke carcinogens. To examine these possibilities, we performed a crossover study in 14 healthy smokers, one-half of whom were African-Americans and one-half whites. Subjects were randomly assigned to smoke mentholated or nonmentholated cigarettes for 1 week, then to cross over to the other type of cigarettes for another week. Subjects were confined to a Clinical Research Center for 3 days of each week, during which time blood levels of nicotine and carbon monoxide were measured throughout the day and an intravenous infusion of deuterium-labeled nicotine and cotinine was administered to determine the rate and pathways of nicotine metabolism. The systemic intake of nicotine and carbon monoxide was, on average, not affected by mentholation of cigarettes. Mentholated cigarette smoking did significantly inhibit the metabolism of nicotine (clearance: 1289 versus 1431 ml/min, two sided, $p = 0.02$). Inhibition of nicotine metabolism occurred both by slower oxidative metabolism to cotinine and by slower glucuronide conjugation. Our data do not support the hypothesis that mentholated cigarette smoking results in a greater absorption of tobacco smoke toxins. Our finding of impaired metabolism of nicotine while mentholated cigarette smoking suggests that mentholated cigarette smoking enhances systemic nicotine exposure.

COMMENTS: This is an expansion of previous research where the authors have shown that African-Americans metabolize nicotine to its metabolite, cotine, differently as compared to whites. The authors report that when the number of cigarettes smoked per day is controlled, and the cigarettes smoked are in machine-determined yield as well as nicotine content, there is no difference in systemic nicotine and CO intake from smoking mentholated cigarettes compared to nonmentholated cigarettes. The results did not indicate that menthol accelerates nicotine metabolism, thus excluding the possibility that a more rapid metabolism of nicotine might explain a greater risk of intake of smoke and thus a greater carcinogenic risk.

3. Epidemiology of menthol cigarette use -

Giovino GA, Sidney S, Gfroerer JC, O'Malley PM, Allen JA, Richter PA, Cummings KM.
Nicotine Tob Res. 2004 Feb;6 Suppl 1:S67-81.

ABSTRACT: Approximately one-fourth of all cigarettes sold in the United States are mentholated. An understanding of the consequences, patterns, and correlates of menthol cigarette use can guide the development and implementation of strategies to reduce smoking prevalence and smoking-attributable morbidity and mortality. This paper summarizes the literature on the health effects of mentholated cigarettes and describes various patterns of use as indicated by consumption and survey data from the United States and other nations. The epidemiological literature on menthol cigarettes and cancer risk is inconclusive regarding whether these cigarettes confer a risk for cancer above that

of nonmentholated varieties. Available data indicate that mentholated cigarettes are at least as dangerous as their nonmentholated counterparts. In addition, because mentholation improves the taste of cigarettes for a substantial segment of the smoking population and appears to mask disease symptoms, this additive may facilitate initiation or inhibit quitting. Menthol market share is high in the Philippines (60%), Cameroon (35%-40%), Hong Kong (26%), the United States (26%), and Singapore (22%). Newport has become the leading menthol brand in the United States. Surveys from four nations indicate that menthol use among adult smokers is more common among females than males. Among U.S. smokers, 68.9% of Blacks, 29.2% of Hispanics, and 22.4% of Whites reported smoking a mentholated variety. Research is needed to better explain factors that may influence menthol preference, such as marketing, risk perceptions, brand formulation, and taste preferences. Such research would guide the development of potentially more effective programs and policies.

COMMENTS: This paper summarizes the literature on the health effects of mentholated cigarettes and describes various patterns of use as indicated by consumption and survey data from the United States and other nations. The epidemiological literature on menthol cigarettes and cancer risk is inconclusive regarding whether these cigarettes confer a risk for cancer above that of nonmentholated varieties.

4. Adolescent menthol smokers: Will they be a harder target for cessation? -

Eric T. Moolchan
Nicotine & Tobacco Research Volume 6, Supplement 1 (February 2004) S93–S95

ABSTRACT: Menthol smoking may influence the development of tobacco addiction and related health consequences, yet limited data on menthol smoking by youth are available. We assessed usual brand menthol preference by Baltimore-area teenage smokers applying to a smoking cessation study between September 1999 and December 2002. Of a biethnic (Black and White) sample of 593 youths (mean age~15.5¡1.4 years, 51% female, 45% African American), the overwhelming majority (93%) were menthol smokers. Menthol preference rates were highest among African American girls and lowest among White boys. Overall, a statistically significant association was found between ethnicity and menthol preference, x2 (df~1)~19.4, pv.001. This association also was observed separately for girls, x2 (df~1)~9.21, p~.0024, and for boys, x2 (df~1)~9.59, p~.0020. Menthol smoking did not vary with age in either ethnic group. These findings of overwhelming menthol preference in a treatment-seeking sample of adolescents warrant further research on the developmental trajectory, cessation, and health-related impact of menthol smoking by youth.

COMMENTS: This study compared the prevalence of menthol preference of Baltimore adolescents of different genders and ethnicities. The study found an overwhelming preference for menthol cigarettes (93%) in teenagers participating in this study. Both ethnicity and gender were significant factors associated with menthol preference. Menthol preference rates were highest in African Americans and females, and lowest in

white males. The findings of this paper were not relevant to the health effects of menthol as an ingredient in cigarettes.

5. Menthol pharmacology and its potential impact on cigarette smoking behavior -

Karen Ahijevych, Bridgette E. Garrett
Nicotine & Tobacco Research Volume 6, Supplement 1 (February 2004) S17–S28

ABSTRACT: Menthol is the only tobacco additive promoted and advertised by the tobacco industry. Although a considerable body of research has examined the effects of menthol when it is administered alone and unburned, the effects of menthol when burned in cigarette smoke are more complex because it is administered in a matrix of more than 4,000 substances. Therefore, it is difficult to isolate potential pharmacological and toxic effects of menthol when it is administered in a smoke mixture. Menthol properties include cooling and local anesthesia, as well as effects on drug absorption and metabolism, bronchodilation and respiration changes, and electrophysiology. Subjective effects of smoothness and less harshness have been identified as reasons for menthol cigarette smoking, but findings have been inconclusive regarding the effect of menthol on carbon monoxide exposure and smoking topography parameters. Gaps in the research literature and future research areas include the following: (a) What is the role of menthol in tobacco reinforcement and addiction? (b) In the absence of nicotine, is menthol reinforcing? (c) Are the pharmacological and physiological effects of menthol mediated by a menthol-specific receptor or some other central nervous system–mediated action? (d) What are the influences of menthol and menthol metabolism on the metabolic activation and detoxification of carcinogens in tobacco smoke? and (e) Do differences exist in cigarette smoking topography in relation to the interaction of ethnicity, gender, and menthol cigarette preference? Answers to these questions will help to elucidate the function of menthol in cigarettes and its impact on smoking behavior.

COMMENTS: These authors reviewed the current knowledge regarding the impact associated with smoking mentholated cigarettes. In this review, the authors attempted to extrapolate the actions of menthol as a nontobacco additive to its potential pharmacological and physiological effects in cigarettes. The authors provided their response to a number of questions that were related to addiction. CNS mediated effects, interaction with race, sex and cigarette preference were all addressed.

6. Percutaneous penetration enhancers in cigarette mainstream smoke -

Smith CJ, Perfetti TA, Garg R, Martin P, Hansch C.
Food Chem Toxicol. 2004 Jan;42(1):9-15.

ABSTRACT: Percutaneous penetration enhancers (PPEs) are chemicals used to enhance the transdermal delivery of drugs. Fifty-eight of the approximately 150 PPEs used for the transdermal delivery of drugs have been reported in cigarette mainstream smoke (MS). MS is a complex aerosol of minute liquid droplets (termed the particulate phase) suspended within a mixture of gases (CO(2), CO, NO(x), etc.) and semi-volatile

compounds. The gases and many of the semi-volatiles are termed the vapor phase. Twenty-nine of the 58 PPEs have been identified in MS vapor phase, 15 in the particulate phase and 14 in both the vapor and particulate phases. There is a tendency for MS PPEs to be hydrophobic, with 40 of the 58 compounds (69%) being either hydrophobic or strongly hydrophobic, and only 24% being hydrophilic. Many of the 4800 known constituents of MS are hydrophilic and would not be expected to readily cross cell membranes or penetrate tissue when delivered as single compounds. The in vivo effect on biological activity of the juxtaposition within the cigarette smoke aerosol of the large number of hydrophilic constituents with the 58 PPEs is currently unknown. As an initial step in understanding this potential complex interaction, the 58 PPEs in MS have been identified and a number of molecular parameters related to the ability to penetrate tissue have been calculated, including MS concentration, measured and calculated base ten logarithm of the octanol-water partition coefficient (Mlog P and Clog P), molecular volume (MgVol) and calculated molar refractivity (CMR).

COMMENTS: Percutaneous penetration enhancers (PPEs) are used by pharmaceutical industry to enhance delivery of drugs that are poorly absorbed. This paper identifies 58 PPEs, including menthol, found in cigarette mainstream smoke and calculates molecular parameters related to the ability to penetrate tissues for each. The authors concluded that the interaction of PPEs in cigarette mainstream smoke with constituents of smoke aerosol cannot be accurately predicted at this time and warrants the study whole cigarette smoke rather than MS fractions.

POTASSIUM CARBONATE
CAS: 584-08-7

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

RUM AND RUM EXTRACT
CAS: 90604-30-1
CAS: 977089-45-4

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

COCOA, COCOA SHELLS, EXTRACT, DISTILLATE, POWDER, ALKALIZED, ABSOLUTE AND TINCTURE
CAS: 08002-31-1
CAS: 84649-99-0
CAS: 68916-17-6
CAS: 95009-22-6

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

GUAR GUM
CAS: 9000-30-0
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

PRUNE JUICE AND CONCENTRATE
CAS: 90082-87-4
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL ALCOHOL, INCLUDING SDA-4
CAS: 64-17-5

Number of relevant papers: 7

GENERAL COMMENTS:
There are numerous papers on a wide range of health effects of drinking alcohol and/or smoking cigarettes. Exposure to one or both substances is a risk factor for possible colon and gastric cancers, abortions, diseases of the mouth and throat, gastro-reflux disease, olfactory ability etc. Few papers are available that examines the effects of inhaled ethanol. While most of the reports did not involve inhalation of the test substance, they all addressed possible synergy between tobacco and alcohol consumption. All of these studies used high levels of EtOH exposure to produce the reported effects. These studies did not mimic the route of exposure nor concentration of EtOH that would be associated with this ingredient used in cigarettes.

1. Pathology of the olfactory epithelium: Smoking and ethanol exposure -

Vent J, Robinson AM, Gentry-Nielsen MJ, Conley DB, Hallworth R, Leopold DA, Kern RC.
Laryngoscope. 2004 Aug;114(8):1383-8.

ABSTRACT: To investigate the effects of tobacco smoke on the olfactory epithelium. Cigarette smoking has been associated with hyposmia; however, the pathophysiology is poorly understood. The sense of smell is mediated by olfactory sensory neurons (OSNs) exposed to the nasal airway, rendering them vulnerable to environmental injury and death. As a consequence, a baseline level of apoptotic OSN death has been demonstrated even in the absence of obvious disease. Dead OSNs are replaced by the mitosis and maturation of progenitors to maintain sufficient numbers of neurons into adult life. Disruption of this balance has been suggested as a common cause for clinical smell loss. This current study will evaluate the effects of tobacco smoke on the olfactory mucosa, with emphasis on changes in the degree of OSN apoptosis. STUDY DESIGN: A rat model was used to assess the olfactory epithelium after exposure to tobacco smoke. METHODS: Rats were exposed to tobacco smoke alone (for 12 weeks), smoke plus dietary ethanol (for the final 5 weeks), or to neither (control). Immunohistochemical analysis of the olfactory epithelium was performed using an antibody to the active form

of caspase-3. Positive staining for this form of the caspase-3 enzyme indicates a cell undergoing apoptotic proteolysis. RESULTS: Control rats demonstrated a low baseline level of caspase-3 activity in the olfactory epithelium. In contrast, tobacco smoke exposure triggered a dramatic increase in the degree of OSN apoptosis that affected all stages of the neuronal lineage. CONCLUSIONS: These results support the following hypothesis: smell loss in smokers is triggered by increased OSN death, which eventually overwhelms the regenerative capacity of the epithelium.

COMMENTS: This study assessed the degree of olfactory sensory neuron (OSN) apoptosis in rats exposed to tobacco smoke with and without ethanol. The report indicates that apoptosis, as demonstrated by caspase-3 activation, is significant after exposure but there was no additional or synergistic effect on caspase-3 activity with ethanol ingestion. This study has little relevance to ethyl alcohol added to cigarette smoke but the authors suggest that increased apoptotic death of OSNs caused by sinusitis and aging, overwhelms the regenerative capacity of the epithelium mediating clinical olfactory loss.

2. A 2-year follow-up study of cigarette smoking and risk of dementia -

D. Juan, D. H. D. Zhou, J. Li, J. Y. J. Wang, C. Gao and M. Chen
European Journal of Neurology Volume 11 Issue 4 Page 277 - April 2004

ABSTRACT: The report focused on investigating the relationship between cigarette smoking and dementia in elderly people through prospective studies. We did a 2-year follow-up study of elderly people. A total of 2820 participants aged 60 years old and over from six communities of Chongqing agreed to take part. Dementia was diagnosed with MMSE (Mini-Mental State Examination) and DSM-III-R (Diagnostic and Statistical Manual of Mental Disorders). Participants were classified as never smokers, past smokers, and current smokers. During follow-up, we recorded incident cases of dementia. The association of smoking and dementia was investigated using proportional hazards regression analysis. A total of 121 incident cases of dementia were detected, of which 84 (69%) were Alzheimer's disease, 17 (14%) were vascular dementia, and 21(17%) were other dementia. Compared with never smokers, current smokers had an increased risk of Alzheimer's disease (RR = 2.72; 95% CI = 1.63–5.42) and vascular dementia (RR = 1.98; 95% CI = 1.53–3.12) adjusting for age, sex, education, blood pressure, and alcohol intake. Compared with light smokers, the adjusted risk of Alzheimer's disease was significantly increased among smokers with a medium level of exposure (RR = 2.56; 95% CI = 1.65–5.52), with an even higher risk of Alzheimer's disease in the heavy smoking group (RR = 3.03; 95% CI = 1.25–4.02). Smoking was associated with the risk of dementia. This study suggests that both smoking status and amount is associated with dementia.

COMMENTS: This paper describes a follow up to a previous study of the relationship between cigarette smoking and cognitive impairment among elderly people in China. Current smoking increased the risk of dementia even after adjusting for other risk factors such as age, sex, education, blood pressure and alcohol intake. However, the risk of

Alzheimer's disease and other forms of dementia was not associated with past smoking amount. The results of this study were not relevant to the health effects of ethyl alcohol as an ingredient in cigarettes.

3. Risk factors for oral and pharyngeal cancer in young adults

Rodriguez T, Altieri A, Chatenoud L, Gallus S, Bosetti C, Negri E, Franceschi S, Levi F, Talamini R, La Vecchia C.
Oral Oncol. 2004 Feb;40(2):207-13.

ABSTRACT: Mortality from oral cancer has been rising in the young in several areas of the world until the early 1990s. We analyzed data from two case-control studies from Italy and Switzerland including 137 cases of oral and pharyngeal cancer below age 46 and 298 hospital controls. The multivariate odds ratios (OR) were 20.7 for heavy smokers and 4.9 for heavy drinkers. The combination of high tobacco and alcohol consumption led to an OR of over 48. Body mass index (OR=0.28, for the highest tertile), high consumption of coffee (OR=0.25), fresh vegetables (OR=0.39), fruit (OR=0.73) and beta-carotene (OR=0.48) were inversely related to risk. Tobacco accounted for 77% of all cancer cases in this population, alcohol for 52%, low vegetable consumption for 52%, and the combination of the three factors for 85%.

COMMENTS: The authors examined the data from two large case-control studies of oral and pharyngel cancer. This report is not relevant to inhaled ethanol since the authors' conclusions are based on use of very high levels of alcohol and an exposure route that did not mimic inhalation. However, the authors' statements regarding the risk for oral/pharyngeal cancers and smoking may be of interest. Heavy consumption of both tobacco smoke and alcohol may result in an over 48-fold increase in health risk in young people. This tobacco-related risk substantially declines within a few years and was not substantially elevated after 5 years of stopping smoking.

4. Desensitization of PKA-stimulated ciliary beat frequency in an ethanol-fed rat model of cigarette smoke exposure -

Wyatt TA, Gentry-Nielsen MJ, Pavlik JA, Sisson JH.
Alcohol Clin Exp Res. 2004 Jul;28(7):998-1004

ABSTRACT: Our previous studies have shown that the ciliary beat frequency (CBF) of cultured ciliated airway epithelial cells exposed to chronic ethanol fails to increase in response to beta-agonist stimulation. This loss of the ciliary "flight response" correlates with an ethanol-mediated desensitization of adenosine 3':5'-cyclic monophosphate-dependent protein kinase (PKA), a known regulatory component of CBF stimulation. We hypothesized that a similar ethanol-mediated desensitization of CBF would occur in vivo. METHODS: Sprague Dawley rats were fed a liquid diet containing various concentrations of ethanol for 1 or 5 weeks. Half were exposed to cigarette smoke for 12 weeks and half were sham exposed. Animals were killed and tracheal epithelial cells analyzed for CBF and PKA activity. RESULTS: Baseline CBF (approximately 6 Hz) was

unchanged in tracheal epithelial cells of rats consuming diets containing 0-36% ethanol for 5 weeks. Isoproterenol stimulated CBF to 12 to 13 Hz in the tracheal epithelial cells of control rats not administered ethanol. However, isoproterenol stimulation of CBF was blunted to 7.5 Hz in rats eating a 26% ethanol diet, and there was no stimulation of CBF in rats fed a diet containing 36% ethanol. Similarly, isoproterenol stimulated a 2- to 3-fold increase in PKA activity in control rats, but this PKA response to isoproterenol was blunted in rats fed increasing concentrations of ethanol. No isoproterenol-stimulated PKA response was observed in rats fed 36% ethanol. No ethanol-induced changes in cyclic guanosine monophosphate-dependent protein kinase or protein kinase C were observed in the rats' tracheal epithelial cells. Cigarette smoke exposure slightly elevated baseline CBF and lowered the ethanol consumption level for isoproterenol-desensitization of CBF and PKA activation to 16%. No isoproterenol desensitization was observed after 1 week of alcohol feeding. Furthermore, 36% ethanol-feeding for 1 week stimulated rat tracheal CBF and PKA. CONCLUSION: These data demonstrate that in vivo administration of ethanol to rats results in decreased ciliary beating and the desensitization of PKA. This suggests a mechanism for mucociliary clearance dysfunction in alcoholics.

COMMENTS: These authors used a rat model to study the combined effects of smoking and ingestion of EtOH to examine the role that smoking has in alcohol-related lung disease. Chronic EtOH use results in desensitization of B-agonist stimulated ciliary beat frequency (CBF), both *in vivo* and *in vitro*, but short term exposure to EtOH does not. Combining cigarette smoke exposure with ethanol further decreases CBF. It is interesting that smoke exposure alone elevated CBF.

5. Percutaneous penetration enhancers in cigarette mainstream smoke.

Smith CJ, Perfetti TA, Garg R, Martin P, Hansch C.
Food Chem Toxicol. 2004 Jan;42(1):9-15.

ABSTRACT: Percutaneous penetration enhancers (PPEs) are chemicals used to enhance the transdermal delivery of drugs. Fifty-eight of the approximately 150 PPEs used for the transdermal delivery of drugs have been reported in cigarette mainstream smoke (MS). MS is a complex aerosol of minute liquid droplets (termed the particulate phase) suspended within a mixture of gases (CO(2), CO, NO(x), etc.) and semi-volatile compounds. The gases and many of the semi-volatiles are termed the vapor phase. Twenty-nine of the 58 PPEs have been identified in MS vapor phase, 15 in the particulate phase and 14 in both the vapor and particulate phases. There is a tendency for MS PPEs to be hydrophobic, with 40 of the 58 compounds (69%) being either hydrophobic or strongly hydrophobic, and only 24% being hydrophilic. Many of the 4800 known constituents of MS are hydrophilic and would not be expected to readily cross cell membranes or penetrate tissue when delivered as single compounds. The in vivo effect on biological activity of the juxtaposition within the cigarette smoke aerosol of the large number of hydrophilic constituents with the 58 PPEs is currently unknown. As an initial step in understanding this potential complex interaction, the 58 PPEs in MS have been identified and a number of molecular parameters related to the ability to penetrate tissue have been calculated, including MS concentration, measured and calculated base ten

logarithm of the octanol-water partition coefficient (Mlog P and Clog P), molecular volume (MgVol) and calculated molar refractivity (CMR).

COMMENTS: Percutaneous penetration enhancers (PPEs) are used by pharmaceutical industry to enhance delivery of drugs that are poorly absorbed. This paper identifies 58 PPEs, including ethanol, found in cigarette mainstream smoke. The molecular parameters related to the ability to penetrate tissues were calculated for each. The authors conclude that the interaction of PPEs in cigarette mainstream smoke with constituents of smoke aerosol cannot be accurately predicted at this time and warrants the study of whole cigarette smoke rather than MS fractions.

6. Subchronic inhalation studies of complex fragrance mixtures in rats and hamsters

Fukayama MY, Easterday OD, Serafino PA, Renskers KJ, North-Root H, Schrankel KR.
Toxicol Lett. 1999 Dec 20;111(1-2):175-87.

ABSTRACT: Users of consumer products are invariably and intentionally exposed to complex mixtures in such products. With finished fragrance products, these mixtures may represent 100 or more fragrance raw materials (FRMs). The objective of the described studies was to evaluate the safety of finished fragrance products via the inhalation route. In total, the finished products contained approximately 100 FRMs at concentrations of 1% or greater. Major FRMs evaluated included benzyl acetate, coumarin, hydroxycitronellal, musk ketone, 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethyl-cyclopenta-gamma-2-be nzopyran (HHCB) and phenyl ethyl alcohol. Groups of rats or hamsters were exposed by inhalation (whole body) to the mixtures at 5, 9 or 50 mg/m3 for 4 h per day, 5 days per week for 6 or 13 weeks. For each of the fragrance products, the doses used generally represented a ten- to 100-fold exaggeration of levels expected to be achieved during typical use by consumers. With one exception, the fragrances were aerosolized prior to introduction into the inhalation chamber. The exception product was formulated with a propellant, packaged in a pressurized container and expelled with an automated actuator. In all studies, chamber concentrations of fragrance were monitored. Particle sizes ranged from 0.5 to 7.5 microm, depending on the study. Subchronic exposure to all fragrance mixtures resulted in no toxicologically significant effects on animal survival, behavior, body weights or weight gains, organ weights, or in hematology, clinical chemistry, or urinalysis parameters. No gross pathological or histopathological findings related to test material exposures were observed. These studies support the conclusions that the fragrance mixtures would not pose a hazard to product users based on repeated and exaggerated inhalation exposures of animals.

COMMENTS: This paper presents results from subchronic inhalation studies of fragrance mixtures in rats and hamsters. Eight fragrance products were tested, which include approximately 200 components, including ethyl alcohol (126 $\mu g/m^3$). No toxicological effects were identified from exposure to the fragrance mixtures at levels 10- to 100 fold greater than expected human exposure conditions for 6 or 13 weeks. The

results are consistent with those earlier studies of Gaworski 1998 that exposed rats to mixtures of flavor ingredients in a subchronic smoke inhalation study.

7. In utero exposure to tobacco and alcohol modifies neurobehavioral development in mice offspring: consideration a role of oxidative stress

Li Y, Wang H, Li JF.
Pharmacol Res 2004; 49: 467-473

ABSTRACT: Objective: To determine whether in utero tobacco and alcohol exposure induces long-term neurobehavioral alterations and whether oxidative stress/damage is a possible causal factor. Methods: Gravid mice were subjected to tobacco smoking and alcohol consumption. Their offspring were subsequently evaluated in developmental and behavioral tests. Antioxidative enzymes and erythrocyte membrane fluidity of adult offspring were measured. Results: The intrauterine tobacco and alcohol exposure has resulted in significant reduced postnatal body and organ weights accompanied by reduced gestational body weight gain in their mothers. Such exposure also induced remarkable developmental delay in neonatal reflexes and notable behavioral deficit in adulthood, namely reduced motive coordination and locomotor activity as well as impaired learning and memory abilities. Furthermore, the formation of malondialdehyde (MDA) increased significantly whereas the activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-PX), catalase (Cat) and glutathione S-transferases (GST) decreased in the cerebral cortex and liver of prenatal intoxicated offspring. The embryonic intoxication also markedly reduced erythrocyte membrane fluidity in offspring. Conclusion: Our study shows the long-term neurotoxicity associated with prenatal tobacco and alcohol exposure, and suggests that the deleterious outcome may be in relation to increased free radicals formation and oxidative stress.

COMMENTS: Pregnant mice were exposed to cigarette smoke and wine in order to examine the prenatal effects of the combined substances. Significant reductions in body weight and delayed neurobehavioral development were observed in the pups of the treated mice. The effects appeared to be long-lasting and related to reductions in the enzyme-mediated antioxidant system. However, this paper was not directly relevant to the health effects of ethyl alcohol as an ingredient in cigarettes.

LICORICE ROOT, FLUID EXTRACT AND POWDER
CAS: 68916-91-6
CAS: 08008-94-4
CAS: 97676-23-8

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

AMMONIUM PHOSPHATE DIBASIC (DIAMMONIUM PHOSPHATE)
CAS: 7783-28-0

Number of relevant papers: 1

1. The effect of tobacco blend additives on the retention of nicotine and solanesol in the human respiratory tract and on subsequent plasma nicotine concentrations during cigarette smoking -

Alan K. Armitage, Michael Dixon,* Barrie E. Frost, Derek C. Mariner,* and Neil M. Sinclair
Chem. Res. Toxicol., 17 (4), 537 -544

ABSTRACT: The influence of the tobacco additives diammonium hydrogen phosphate (DAP) and urea on the delivery and respiratory tract retention of nicotine and solanesol and on the uptake of nicotine into venous blood was investigated in 10 smokers under mouth-hold and 75 and 500 mL inhalation conditions. Three cigarettes with identical physical specifications were produced from a common lamina tobacco blend. The control cigarette contained nonammoniated reconstituted tobacco sheet (RTS), whereas DAP and other ammonia compounds were added to the RTS of the second cigarette. Urea was added to the tobacco of the third cigarette. The presence of DAP or urea in the test cigarettes did not significantly influence solanesol retention within the mouth during the mouth-hold condition. Nicotine retention within the mouth during the mouth-hold condition was, however, significantly higher for the DAP cigarette (64.3 ± 10.5%) than for the urea (53.3 ± 11.3%) or control cigarette (46.3 ± 8.6%), but this did not result in an increase in nicotine uptake into venous blood. Solanesol retentions during the 75 and 500 mL inhalation volume conditions and nicotine retentions during the 75 mL inhalation volume condition were not significantly different for the three cigarette types. Although the nicotine retention approached 100% with each cigarette type during the 500 mL inhalation condition, the nicotine retention for the urea-treated cigarette (99.6 ± 0.2%) was marginally, but statistically, significant, higher than for the control (99.1 ± 0.5%) and DAP-treated cigarettes (98.8 ± 0.6%). There were no statistically significant differences between the indices of nicotine uptake into venous blood for the three cigarette types in any of the inhalation conditions.

COMMENTS: It has been postulated that certain ammonium compounds when used as a tobacco additive can increase smoke pH thus increasing the transfer of nicotine from tobacco to the smoke and increasing the "addictiveness" of nicotine. This study assesses the retention of nicotine in the respiratory tract and its uptake into the blood system under controlled inhalation conditions. These results do not indicate that the addition of diammonium hydrogen phosphate or urea resulted in an enhanced uptake of nicotine from the respiratory tract into the systemic circulation during smoking. The authors found that most of the nicotine inhaled in cigarette smoke is absorbed irrespective of the

pH and that the pH does not affect bioavailability but instead influences the perceived strength of the cigarette.

AMMONIUM ALGINATE
CAS: 9005-34-9
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

CHOCOLATE AND CHOCOLATE LIQUOR
MAJOR
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

LACTIC ACID
CAS: 50-21-5
CAS: 598-82-3
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

PLUM JUICE, CONCENTRATE AND EXTRACT
CAS: 90082-87-4
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

CAROB BEAN GUM, ABSOLUTE AND EXTRACT
CAS: 9000-40-2
CAS: 84961-45-5
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

FIG JUICE CONCENTRATE AND EXTRACT
CAS: 90028-74-3
CAS: 68916-52-9
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

SORBITOL
CAS: 50-70-4
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

AMMONIUM HYDROXIDE
CAS: 1336-21-6
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

GLUCOSE/ DEXTROSE
CAS: 50-99-7
CAS: 492-62-6

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

UREA
CAS: 57-13-6

Number of relevant papers: 1

1. The effect of tobacco blend additives on the retention of nicotine and solanesol in the human respiratory tract and on subsequent plasma nicotine concentrations during cigarette smoking -

Alan K. Armitage, Michael Dixon,* Barrie E. Frost, Derek C. Mariner,* and Neil M. Sinclair
Chem. Res. Toxicol., 17 (4), 537 -544

ABSTRACT: The influence of the tobacco additives diammonium hydrogen phosphate (DAP) and urea on the delivery and respiratory tract retention of nicotine and solanesol and on the uptake of nicotine into venous blood was investigated in 10 smokers under mouth-hold and 75 and 500 mL inhalation conditions. Three cigarettes with identical physical specifications were produced from a common lamina tobacco blend. The control cigarette contained nonammoniated reconstituted tobacco sheet (RTS), whereas DAP and other ammonia compounds were added to the RTS of the second cigarette. Urea was added to the tobacco of the third cigarette. The presence of DAP or urea in the test cigarettes did not significantly influence solanesol retention within the mouth during the mouth-hold condition. Nicotine retention within the mouth during the mouth-hold condition was, however, significantly higher for the DAP cigarette (64.3 ± 10.5%) than for the urea (53.3 ± 11.3%) or control cigarette (46.3 ± 8.6%), but this did not result in an increase in nicotine uptake into venous blood. Solanesol retentions during the 75 and 500 mL inhalation volume conditions and nicotine retentions during the 75 mL inhalation volume condition were not significantly different for the three cigarette types. Although the nicotine retention approached 100% with each cigarette type during the 500 mL inhalation condition, the nicotine retention for the urea-treated cigarette (99.6 ± 0.2%) was marginally, but statistically, significant, higher than for the control (99.1 ± 0.5%) and DAP-treated cigarettes (98.8 ± 0.6%). There were no statistically significant differences between the indices of nicotine uptake into venous blood for the three cigarette types in any of the inhalation conditions.

COMMENTS: It has been postulated that certain ammonium compounds when used as a tobacco additive can increase smoke pH thus increasing the transfer of nicotine from tobacco to the smoke and increasing the "addictiveness" of nicotine. This study assesses the retention of nicotine in the respiratory tract and its uptake into the blood system under controlled inhalation conditions. These results do not indicate that the addition of

diammonium hydrogen phosphate or urea resulted in an enhanced uptake of nicotine from the respiratory tract into the systemic circulation during smoking. The authors found that most of the nicotine inhaled in cigarette smoke is absorbed irrespective of the pH and that the pH does not affect bioavailability but instead influences the perceived strength of the cigarette.

SODIUM CARBONATE
CAS: 497-19-8

Number of relevant papers: 1

1. Cancer incidence in textile manufacturing workers in Australia

Fritschi L, Lakhani R, Nadon L.
J Occup Health 2004 Nov;46(6):493-6.

ABSTRACT: N/A

COMMENTS: The study was designed to assess the associated of incidence of cancer with the likely exposure to individual chemicals in textile manufacturing workers. There were no significant increases in relative risk of cancer associated with any of the 32 substances assessed, including sodium carbonate, which had a relative risk of 1.55.

FRUCTOSE
CAS: 57-48-7
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

DAVANA OIL
CAS: 8016-03-3
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

LIME OIL
CAS: 68916-84-7
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL 2-METHYLBUTYRATE
CAS: 7452-79-1
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

PEPPERMINT OIL AND ABSOLUTE AND PEPPERMINT OIL TERPENELESS
CAS: 8006-90-4
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

SPEARMINT OIL
CAS: 8008-79-5
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ORANGE OIL AND EXTRACT (SWEET, DISTILLED, TERPENELESS, AND SOUR/BITTER ORANGE OILS)
CAS: 8008-57-9
CAS: 68606-94-0
CAS: 68916-04-1
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

MOLASSES EXTRACT
CAS: 8052-35-5
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

CORIANDER EXTRACT, SEED, AND OIL
CAS: 8008-52-4
CAS: 84775-50-8
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL VANILLIN
CAS: 121-32-4
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

L-MENTHONE
CAS: 14073-97-3
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

VANILLIN
CAS: 121-33-5

Number of relevant papers: 1

1. Mutagens and Sensitizers-An Unequal Relationship? -

A. M. Wolfreys A1 and D. A. Basketter A1
Journal of Toxicology: Cutaneous and Ocular Toxicology Volume 23, Number 3 / 2004 197 – 205.

ABSTRACT: For some years, those involved with the safety assessment of chemicals have in one way or another considered the degree to which data on either skin sensitization potential or on carcinogenicity may inform them on the other endpoint for a particular substance. In this work, we have taken a pragmatic perspective on the question and assessed mutagens, rather than carcinogens, and sensitizers as this better reflects the potential for biological macromolecule interaction. A dataset of 100 substances, the majority of which have come under scrutiny for one reason or another during our own toxicology investigations, was interrogated. We focused on the extent to which results from the primary screen for skin sensitization correlated with the results from the two in vitro tests used as a screen for mutagenicity, namely the bacterial mutation assay and the in vitro chromosome aberration assay. Although there was some concordance between the two endpoints, as standalone methods, neither predicted the other particularly accurately, with 32% showing disagreement. It is probable that there are several critical elements missing from this top level assessment, not least an appreciation of which substances are positive in mutagenicity tests via non genotoxic mechanisms which could seriously impair such a correlation between results from the two different endpoints.

COMMENTS: This paper discusses the relationship between skin sensitizers and carcinogens. Previous data indicate that chemicals that induced allergic contact dermatitis had a 50% chance of being a rodent carcinogen. To investigate this hypothesis the authors examined *in vitro* mutagenicity screening data on 100 chemicals and compared the results with information on skin sensitization potential of these substances. In these comparisons about one-third of the chemicals that were positive in the mutagenicity screen would not be classified as skin sensitizer. Vanillin was mutagenic but was not a skin sensitizer. The author's conclusion was that neither endpoint is a reliable indicator of the other.

CHAMOMILE FLOWER OIL, EXTRACT AND ABSOLUTE
CAS: 8002-66-2
CAS: 8015-92-7

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

CATEGORY: STANDARD INGREDIENTS

1. Percutaneous penetration enhancers in cigarette mainstream smoke -

Smith CJ, Perfetti TA, Garg R, Martin P, Hansch C.
Food Chem Toxicol. 2004 Jan;42(1):9-15.

This paper examines a number of standard ingredients including:

BENZYL ALCOHOL 100-51-6
1,3-BUTANEDIOL 107-88-0
BUTYL ACETATE 123-86-4
CARBON DIOXIDE 124-38-9
ETHYL ACETATE 141-78-6
DECANOIC ACID 334-48-5
BUTYL ALCOHOL (1-BUTANOL) 71-36-3

ABSTRACT: Percutaneous penetration enhancers (PPEs) are chemicals used to enhance the transdermal delivery of drugs. Fifty-eight of the approximately 150 PPEs used for the transdermal delivery of drugs have been reported in cigarette mainstream smoke (MS). MS is a complex aerosol of minute liquid droplets (termed the particulate phase) suspended within a mixture of gases (CO2, CO, NOx, etc.) and semi-volatile compounds. The gases and many of the semi-volatiles are termed the vapor phase. Twenty-nine of the 58 PPEs have been identified in MS vapor phase, 15 in the particulate phase and 14 in both the vapor and particulate phases. There is a tendency for MS PPEs to be hydrophobic, with 40 of the 58 compounds (69%) being either hydrophobic or strongly hydrophobic, and only 24% being hydrophilic. Many of the 4800 known constituents of MS are hydrophilic and would not be expected to readily cross cell membranes or penetrate tissue when delivered as single compounds. The in vivo effect on biological activity of the juxtaposition within the cigarette smoke aerosol of the large number of hydrophilic constituents with the 58 PPEs is currently unknown. As an initial step in understanding this potential complex interaction, the 58 PPEs in MS have been identified and a number of molecular parameters related to the ability to penetrate tissue have been calculated, including MS concentration, measured and calculated base ten logarithm of the octanol-water partition coefficient (Mlog P and Clog P), molecular volume (MgVol)and calculated molar refractivity (CMR).

COMMENTS: Percutaneous penetration enhancers (PPEs) are used by pharmaceutical industry to enhance delivery of drugs that are poorly absorbed. This paper identifies 58 PPEs found in cigarette mainstream smoke and calculates molecular parameters related to the ability to penetrate tissues for each. The authors concluded that the interaction of PPEs in cigarette mainstream smoke with constituents of smoke aerosol cannot be accurately predicted at this time and warrants the study of whole cigarette smoke rather than MS fractions.

ACETANISOLE
CAS: 100-06-1
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ACETIC ACID
CAS: 64-19-7
SEE HIGH MUL'S INGREDIENTS

ACETOIN
CAS: 513-86-0
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ACETOPHENONE
CAS:98-86-2
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ACETYLPYRAZINE (2-)
CAS: 22047-25-2
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

3-ACETYLPYRIDINE (BETA-ACETYLPYRIDINE)
CAS: 350-03-8
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

2-ACETYLTHIAZOLE
CAS: 24295-03-2
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

DL-ALANINE, L-ALANINE
CAS: 302-72-7
CAS: 56-41-7
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ALFALFA EXTRACT
CAS: 84082-36-0
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ALLYL HEXANOATE
CAS: 123-68-2
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

AMMONIUM ALGINATE
CAS: 9005-34-9
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

AMMONIUM HYDROXIDE
CAS: 1336-21-6
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

AMMONIUM PHOSPHATE DIBASIC (DIAMMONIUM PHOSPHATE)
CAS: 7783-28-0
SEE MAJOR INGREDIENTS

AMYL ALCOHOL
CAS: 71-41-0
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

AMYL BUTYRATE
CAS: 540-18-1
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

AMYL FORMATE
CAS: 638-49-3
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

AMYL OCTANOATE
CAS: 638-25-5
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ALPHA-AMYLCINNAMALDEHYDE
CAS: 122-40-7
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

TRANS-ANETHOLE
CAS: 4180-23-8
CAS: 104-46-1

Number of relevant papers: 1

1. Cytotoxic and xenoestrogenic effects via biotransformation of trans-anethole on isolated rat hepatocytes and cultured MCF-7 human breast cancer cells -

Nakagawa Y, Suzuki T.
Biochem Pharmacol. 2003 Jul 1;66(1):63-73.

ABSTRACT: Themetabolism and action of trans-anethole (anethole) and the estrogen-like activity of the compound and its metabolites were studied in freshly isolated rat hepatocytes and cultured MCF-7 human breast cancer cells, respectively. The incubation of hepatocytes with anethole (0.25–2.0 mM) caused a concentration- and time-dependent cell death accompanied by losses of cellular ATP and adenine nucleotide pools. Anethole at a weakly toxic level (0.5 mM) was metabolized to 4-methoxycinnamic acid (4MCA), 4-hydroxy-1-propenylbenzene (4OHPB), and the monosulfate conjugate of 4OHPB; the levels of 4OHPB sulfate and 4MCA reached approximately 20 and 200 mM within 2 hr,respectively, whereas that of free unconjugated 4OHPB was less than approximately 0.5 mM. At amoderately toxic concentration (1.0 mM), unconjugated4OHPBreached approximately 10 mM, followed by abrupt loss of 30-phosphoadenosine 50-phosphosulphate (PAPS). Based on cell viability and adenine nucleotide levels, 4OHPB was more toxic than anethole and 4MCA. The addition of 2,6-dichloro-4-nitrophenol (50 mM), an inhibitor of sulfotransferase, enhanced the anethole-induced cytotoxicity associated with losses of ATP, PAPS, and 4OHPB sulfate, and symmetrically increased the unconjugated 4OHPB concentration. 4OHPB as well as diethylstilbestrol (DES) and bisphenol A (BPA), which are known xenoestrogenic compounds, competitively displaced 17b-estradiol bound to the estrogen receptor a in a concentration-dependent manner; IC50 values of these compounds were approximately 1 _ 10_5, 1 _ 10_8 and 5 _ 10_5 M, respectively. 4OHPB also caused a concentration (10_8 to 10_6 M)-dependent proliferation ofMCF-7 cells, whereas neither anethole nor 4MCA(10_9 to 10_5 M) affected cell proliferation. However, at higher concentrations (>10_4 M), 4OHPB rather than anethole and 4MCAwas cytotoxic. These results suggest that the biotransformation of anethole induces a cytotoxic effect at higher concentrations in rat hepatocytes and an estrogenic effect at lower concentrations in MCF-7 cells based on the concentrations of the hydroxylated intermediate, 4OHPB.

COMMENTS: The toxicity of trans-anethole and its metabolites were measured in rat hepatocytes and MCF-7 cells. Concentration-dependent and time-dependent cytotoxicity was observed in rat hepatocytes at anethole exposures ranging from 0.25 – 2. mM. The hydroxylated metabolite, 4-hydroxy-1-propenylbenzene (4OHPB) and not the parent compound, induced cytotoxic and estrogenic effects. Treatment with 4OHPB resulted in decreased cell viability and loss of intracellular levels of ATP and total adenine

nucleotide pools in hepatocytes. Estrogenic activity of 4OHPB was observed based on a proliferative assay of estrogen-responsive human breast cancer cells and a concentration-dependent displacement of 17β-estradiol bound to ERα. This study suggests that anethole may become cytotoxic and estrogenic via biotransformation and highlights the importance of using *in vivo* experiments to assess anethole toxicity.

ANGELICA ROOT EXTRACT AND OIL
CAS: 84775-41-7

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ANISE STAR OIL
CAS: 8007-70-3

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ANISYL ACETATE
CAS: 104-21-2

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

APPLE JUICE CONCENTRATE, ESSENCE AND EXTRACT
CAS: 85251-63-4

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

L-ARGININE
CAS: 74-79-3

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ASCORBIC ACID
CAS: 50-81-7

Number of relevant papers: 1

1. Cigarette smoke effects on salivary antioxidants and oral cancer - Novel concepts

Rafael M. Nagler MD DMD PhD and Abraham Z. Reznick PhD
Isr Med Assoc J 2004 Nov;6:691-4

ABSTRACT: Oral squamous cell carcinoma is the most common malignancy of the head and neck, with a worldwide incidence of over 300,000 new cases annually [1]. The disease is characterized by a high rate of morbidity and mortality (about 50%) [1±4]. The major inducer of oral SCC is exposure to tobacco, considered to be responsible for

50±90% of cases worldwide [5±7]. The incidence of oral SCC in cigarette smokers is four to seven times higher than in non-smokers; when alcohol is also consumed this incidence is even higher. Moreover, compared with non-smokers, the higher cigarette smoke-related risk for oral SCC is manifested by a reduction in the mean age of development of the disease by 15 years [8,9]. The ``field cancerization" concept is the currently accepted explanation for the carcinogenic effect of cigarette smoke on oral mucosa [10]. According to this theory, there is a constant and direct attack of various cigarette smoke reagents on the oral epithelial cells, which gradually accumulate and cause a step-wise malignant transformation. It has been suggested that free radicals, reactive oxygen species and reactive nitrogen species in the inhaled cigarette smoke induce this gradually evolving process, initially expressed by dysplastic lesions of the mucosa, are then trans-formed into in situ carcinoma lesions and eventually result in full-blown infiltrating and metastasizing oral SCC. Further credence for the suggested role of free radicals in the pathogenesis of evolving oral SCC is found in a recent study [11] demonstrating that ROS, such as hydroxyl radical, are formed in the human oral cavity during areca quid chewing, and that the activity might cause oxidative DNA damage to the surrounding tissues. In this respect the salivary anticarcinogenic capacity, which has only recently been recognized, may be based on its antioxidant system.

COMMENTS: Aspects of the salivary defense system are discussed including antioxidant enzymes (peroxidase and superoxide dismutase) and molecules such as uric acid and ascorbic acid. Cigarette smoke has been shown to reduce activity of salivary antioxidant enzymes, but not antioxidant molecules. Salivary peroxidase activity was not affected by exposure to purified aldehydes, nicotine or ascorbic acid, but appeared to be affected by hydrogen cyanide exposure. The enzyme activity returned to pre-smoking levels after 30 minutes, presumably due to the secretion of new saliva into the oral cavity.

L-ASPARTIC ACID
CAS: 56-84-8

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

BALSAM PERU AND OIL
CAS: 8007-00-9

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

BEESWAX RESINOID AND ABSOLUTE
CAS: 8006-40-4
CAS: 8012-89-3

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

BEET JUICE CONCENTRATE
CAS: 89957-90-4

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

BENZALDEHYDE
CAS: 100-52-7

SEE HIGH MUL'S INGREDIENTS

BENZALDEHYDE GLYCERYL ACETAL
CAS: 1319-88-6

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

BENZOIC ACID
CAS: 65-85-0

Number of relevant papers: 1

1. Controversies in toxicology assessing food additive toxicity using a cell model -

Stefanidou M; Alevisopoulos G; Chatziioannou A; Koutselinis
Veterinary and Human Toxicology, 2003 , 45/2 (103-105)

ABSTRACT: Food additives are widely used for technological purposes and their presence is often substantial daily diet. They have also been accused for various toxic reactions in humans. The toxicity of the food color tartrazine, the preservatives sodium nitrate and sodium benzoate, and the antioxidant BHT, was studied using the protozoan Tetrahymena pyriformis as a toxicological model. The 4 food additives were added to Tetrahymena cultures and DNA content of the protozoan nuclei measured by an image analysis system. These food additives caused a statistically significant increase in DNA content suggesting stimulation of the mitotic process. This system may contribute to the investigation of the cellular action of food additives, since mitogenic stimuli substantiallly alter susceptibility to chemical carcinogenesis. (32 References)

COMMENTS: These investigators tested the cytotoxic effect of 4 food additives, including sodium benzoate using a protozoan assay. Sodium benzoate activity is dependent on the concentration of undissociated benzoic acid. Some individuals exhibit allergy to benzoates. All four of the additives produced significant increase in DNA synthesis in protozoa macronucleus. The authors suggest that when this effect occurs, other cell activities are also depressed such as phagocytosis.

BENZOIN, RESIN, RESINOID, TINCTURE, GUM AND ABSOLUTE
CAS: 9000-05-9
CAS: 84012-39-5
CAS: 9000-72-0
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

BENZYL ALCOHOL
CAS: 100-51-6

Number of relevant papers: 3

1. Anti-estrogenic activity of fifty chemicals evaluated by in vitro assays

Joohee Jung , Kunie Ishida and Tsutomu Nishihara ,
Life Sciences Volume 74, Issue 25 , 7 May 2004, Pages 3065-3074

ABSTRACT: We examined the anti-estrogenic activity of 50 chemicals by the yeast two-hybrid assay and detected the activity of hexachlorophene, pentachlorophenol, and vitamin K3 (menadione), in that order. These chemicals were also
observed to inhibit the transcriptional activity of 17h-estradiol in a reporter gene assay system using MCF-7 cells, estrogen receptor-positive breast cancer cells, and to bind directly to estrogen receptor a in a competitive binding assay system, although the order of the activity was slightly different among the 3 assays. These findings suggested that three of fifty chemicals could inhibit estrogen activity by competitive binding with 17h-estradiol to the estrogen receptor.

COMMENTS: The inhibitory effect of various chemicals against 17β-estradiol was assessed using the yeast two-hybrid assay. Fifty chemicals, including benzyl alcohol were tested in a range from 10^{-3} to 10^{-9} M. Only three chemicals showed inhibition of estrogenic activity. No anti-estogenic activity was reported for benzyl alcohol within the range of concentrations tested.

2. Neurologic issues with solvents

Rutchik JS, Wittman RI.
Clin Occup Environ Med. 2004 Nov;4(4):621-56, v-vi.

ABSTRACT: Organic solvents are a chemical class of compounds that are used routinely in commercial industries. They possess a low molecular weight, share a similar structure, lipophilicity, and volatility, and theyexist in liquid at room temperature. They may be grouped further into aliphatic compounds that exist in chain form, such as n-hexane,and aromatic compounds that exist in a 6-carbon ring form, such as benzene or xylene. Aliphatics and aromatics may contain a substituted halogen element and may be referred to as halogenated hydrocarbons, such as perchloroethylene, trichloroethylene,

and crbon tetrachloride. Alcohols, ketones, glycols, esters,ethers, aldehydes, and pyridines exist due to substitutions for a hydrogen group.

COMMENTS: This is a well-documented review of neurologic effects from exposure to a variety of solvents. The only discussion focusing on benzyl alcohol was that it was shown to block neuronal action potentials reversibly *in vitro* and exposure of rat nerve roots results in scattered demyelination and axonal degeneration.

3. Subchronic inhalation studies of complex fragrance mixtures in rats and hamsters

Fukayama MY, Easterday OD, Serafino PA, Renskers KJ, North-Root H, Schrankel KR.
Toxicol Lett. 1999 Dec 20;111(1-2):175-87.

ABSTRACT: Users of consumer products are invariably and intentionally exposed to complex mixtures in such products. With finished fragrance products, these mixtures may represent 100 or more fragrance raw materials (FRMs). The objective of the described studies was to evaluate the safety of finished fragrance products via the inhalation route. In total, the finished products contained approximately 100 FRMs at concentrations of 1% or greater. Major FRMs evaluated included benzyl acetate, coumarin, hydroxycitronellal, musk ketone, 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethyl-cyclopenta-gamma-2-be nzopyran (HHCB) and phenyl ethyl alcohol. Groups of rats or hamsters were exposed by inhalation (whole body) to the mixtures at 5, 9 or 50 mg/m3 for 4 h per day, 5 days per week for 6 or 13 weeks. For each of the fragrance products, the doses used generally represented a ten- to 100-fold exaggeration of levels expected to be achieved during typical use by consumers. With one exception, the fragrances were aerosolized prior to introduction into the inhalation chamber. The exception product was formulated with a propellant, packaged in a pressurized container and expelled with an automated actuator. In all studies, chamber concentrations of fragrance were monitored. Particle sizes ranged from 0.5 to 7.5 microm, depending on the study. Subchronic exposure to all fragrance mixtures resulted in no toxicologically significant effects on animal survival, behavior, body weights or weight gains, organ weights, or in hematology, clinical chemistry, or urinalysis parameters. No gross pathological or histopathological findings related to test material exposures were observed. These studies support the conclusions that the fragrance mixtures would not pose a hazard to product users based on repeated and exaggerated inhalation exposures of animals.

COMMENTS: This paper presents results from subchronic inhalation studies of fragrance mixtures in rats and hamsters. Eight fragrance products were tested, which include approximately 200 components, including benzyl alcohol (52 $\mu g/m^3$). No toxicological effects were identified from exposure to the fragrance mixtures at levels 10- to 100 fold greater than expected human exposure conditions for 6 or 13 weeks. The results are consistent with those of earlier studies of Gaworski 1998 that exposed rats to mixtures of flavor ingredients in a subchronic smoke inhalation study.

BENZYL BENZOATE
CAS: 120-51-4

Number of relevant papers: 2

1. Subchronic inhalation studies of complex fragrance mixtures in rats and hamsters

Fukayama MY, Easterday OD, Serafino PA, Renskers KJ, North-Root H, Schrankel KR.
Toxicol Lett. 1999 Dec 20;111(1-2):175-87.

ABSTRACT: Users of consumer products are invariably and intentionally exposed to complex mixtures in such products. With finished fragrance products, these mixtures may represent 100 or more fragrance raw materials (FRMs). The objective of the described studies was to evaluate the safety of finished fragrance products via the inhalation route. In total, the finished products contained approximately 100 FRMs at concentrations of 1% or greater. Major FRMs evaluated included benzyl acetate, coumarin, hydroxycitronellal, musk ketone, 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethyl-cyclopenta-gamma-2-be nzopyran (HHCB) and phenyl ethyl alcohol. Groups of rats or hamsters were exposed by inhalation (whole body) to the mixtures at 5, 9 or 50 mg/m3 for 4 h per day, 5 days per week for 6 or 13 weeks. For each of the fragrance products, the doses used generally represented a ten- to 100-fold exaggeration of levels expected to be achieved during typical use by consumers. With one exception, the fragrances were aerosolized prior to introduction into the inhalation chamber. The exception product was formulated with a propellant, packaged in a pressurized container and expelled with an automated actuator. In all studies, chamber concentrations of fragrance were monitored. Particle sizes ranged from 0.5 to 7.5 microm, depending on the study. Subchronic exposure to all fragrance mixtures resulted in no toxicologically significant effects on animal survival, behavior, body weights or weight gains, organ weights, or in hematology, clinical chemistry, or urinalysis parameters. No gross pathological or histopathological findings related to test material exposures were observed. These studies support the conclusions that the fragrance mixtures would not pose a hazard to product users based on repeated and exaggerated inhalation exposures of animals.

COMMENTS: This paper presents results from subchronic inhalation studies of fragrance mixtures in rats and hamsters. Eight fragrance products were tested, which include approximately 200 components, including benzyl benzoate (3 - 694 µg/m3). No toxicological effects were identified from exposure to the fragrance mixtures at levels 10- to 100 fold greater than expected human exposure conditions for 6 or 13 weeks. The results are consistent with those of earlier studies of Gaworski 1998 that exposed rats to mixtures of flavor ingredients in a subchronic smoke inhalation study.

2. Inhibitory effects of the essential oil from SuHeXiang Wan on the central nervous system after inhalation -

Koo BS, Lee SI, Ha JH, and Lee DU
Biological & Pharmaceutical Bulletin Vol. 27 (2004), No. 4, 515-519.

ABSTRACT: The present study was performed to evaluate the central nervous system inhibitory effects of the essential oil from SuHeXiang Wan (Storax pill), a prescription usually used for treating epilepsy in traditional Chinese medicine, on fragrance inhalation (aroma therapy). Preinhalation of the fragrance oil markedly delayed the appearance of pentylenetetrazole-induced convulsion, but showed weak activities on picrotoxin- and strychnine-induced convulsions, which implies this drug may inhibit the convulsion by GABAergic neuromodulation. This essential oil inhibited the binding of [3H]Ro15-1788, a selective antagonist for the benzodiazepine receptor and also the binding of [3H]flunitrazepam, a selective agonist for the receptor, in the presence of g-aminobutyric acid (GABA) and NaCl, showing a positive GABA shift, which suggested the strong possibility of the agonistic activity of the essential oil to the GABA/benzodiazepine receptor complex in rat cerebral cortices. Furthermore, inhalation inhibited the activity of GABA transaminase as the inhalation period was lengthened. The GABA level was signifi- cantly increased and glutamate content was significantly decreased in mouse brain by preinhalation of the essential oil. The above results suggest that the anticonvulsive effect of this essential oil can also originate from the enhancement of GABA level in the mouse brain, because convulsion depends partially on GABA concentration which can be properly preserved by inhibiting GABA transaminase. Fragrance inhalation progressively prolonged the pentobarbital-induced sleeping time as inhalation time was lengthened and inhibited brain lipid peroxidation, to which the anticonvulsive action is attributed; this also supported the above results, confirming the inhibitory effects of the essential oil of SuHeXiang Wan on the CNS via the GABAergic system.

COMMENTS: Fragrance inhalation of essential oils which make up Chinese medicinal prescriptions was shown to possess anticonvulsive and sedative properties in mouse experiments. Anticonvulsive effect of the essential oils was attributed to enhanced GABA levels and decreased lipid peroxidation in mouse brain. Benzyl benzoate was one of 10 compounds detected in the essential oils, and accounted for only 5.4% of the content of the mixture. Therefore, the relevance of this study to the health effects of benzyl benzoate as an ingredient in cigarettes is minimal.

BENZYL CINNAMATE (PROPENIC ACID, 3-PHENYL, PHENYLMETHYL ESTER,2-)
CAS: 103-41-3

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

BENZYL PHENYLACETATE
CAS: 102-16-9
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

BENZYL PROPIONATE
CAS: 122-63-4
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

BORNYL ACETATE
CAS: 76-49-3
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

1,3-BUTANEDIOL
CAS: 107-88-0
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

2, 3-BUTANEDIONE (DIACETYL)
CAS: 431-03-8
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

BUTANOIC ACID, 3-METHYL-, 4-METHYLPHENYL ESTER (PARA-TOLYL 3-METHYLBUTYRATE) (P-TOLYL ISOVALERATE)
CAS: 55066-56-3
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

BUTTER, BUTTER ESTERS, AND BUTTER OIL
CAS: 91745-88-9
CAS: 97926-23-3
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

BUTYL ACETATE
CAS: 123-86-4
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

BUTYL ALCOHOL (1-BUTANOL)
CAS: 71-36-3

Number of relevant papers: 1

1. Chemically induced renal tubule tumors in the laboratory rat and mouse: Review of the NCI/NTP database and categorization of renal carcinogens based on mechanistic information

Edward Lock; Gordon Hard
Critical Reviews in Toxicology, Volume 34, Number 3, May-June 2004, pp. 211-299(89)

Abstract: The incidence of renal tubule carcinogenesis in male and female rats or mice with 69 chemicals from the 513 bioassays conducted to date by the NCI/NTP has been collated, the chemicals categorized, and the relationship between carcinogenesis and renal tubule hyperplasia and exacerbation of the spontaneous, age-related rodent disease chronic progressive nephropathy (CPN) examined. Where information on mechanism or mode of action exists, the chemicals have been categorized based on their ability to directly or indirectly interact with renal DNA, or on their activity via epigenetic pathways involving either direct or indirect cytotoxicity with regenerative hyperplasia, or exacerbation of CPN. Nine chemicals were identified as directly interacting with DNA, with six of these producing renal tubule tumors at high incidence in rats of both sexes, and in some cases also in mice. Ochratoxin A was the most potent compound in this group, producing a high tumor incidence at very low doses, often with metastasis. Three chemicals were discussed in the context of indirect DNA damage mediated by an oxidative free radical mechanism, one of these being from the NTP database. A third category included four chemicals that had the potential to cause DNA damage following conjugation with glutathione and subsequent enzymatic activation to a reactive species, usually a thiol-containing entity. Two chemicals were allocated into the category involving a direct cytotoxic action on the renal tubule followed by sustained compensatory cell proliferation, while nine were included in a group where the cell loss and sustained increase in renal tubule cell turnover were dependent on lysosomal accumulation of the male rat-specific protein, 2-globulin. In a sixth category, morphologic evidence on two chemicals indicated that the renal tumors were a consequence of exacerbated CPN. For the remaining chemicals, there were no pertinent data enabling assignment to a mechanistic category. Accordingly, these chemicals, acting through an as yet unknown mechanism, were grouped as either being associated with an enhancement of CPN mechanism, were grouped as either being associated with an enhancement of CPN (category 7, 16 chemicals), or not associated with enhanced CPN (category 8, 4 chemicals). A ninth category dealt with 11 chemicals that were regarded as producing increases in renal tubule tumors that did not reach statistical significance. A 10th category discussed 6 chemicals that induced renal tumors in mice but not in rats, plus 8 chemicals that produced a low incidence of renal tubule tumors in mice that did not reach statistical significance. As more mechanistic data are generated, some chemicals will inevitably be placed in different groups, particularly those from categories

7 and 8. A large number of chemicals in the series exacerbated CPN, but those in category 7 especially may be candidates for inclusion in category 6 when further information is gleaned from the relevant NTP studies. Also, new data on specific chemicals will probably expand category 5 as cytotoxicity and cell regeneration are identified as obligatory steps in renal carcinogenesis in more cases. Additional confirmatory outcomes arising from this review are that metastases from renal tubule tumors, while encountered with chemicals causing DNA damage, are rare with those acting through an epigenetic pathway, with the exception being fumonisin B1; that male rats and mice are generally more susceptible than female rats and mice to chemical induction of renal tubule tumors; and that a background of atypical tubule hyperplasia is a useful indicator reflecting a chemically associated renal tubule tumor response. With respect to renal tubule tumors and human risk assessment, chemicals in categories 1 and 2, and possibly 3, would currently be judged by linear default methods; chemicals in category 4 (and probably some in category 3) as exhibiting a threshold of activity warranting the benchmark approach; and those in categories 5 and 6 as representing mechanisms that have no relevance for extrapolation to humans.

COMMENTS: This paper provides a review of 69 chemicals tested in the National Cancer Institute / National Toxicology Program (NCI/NTP) carcinogenicity bioassay database including butyl alcohol. The selected chemicals are those that have shown an association with renal tubule tumors in rat and/or mouse. Butyl alcohol was placed in category 5, considered "chemicals inducing renal tumors via indirect cytotoxicity and sustained tubule cell regeneration associated with $\alpha_{2\mu}$-globulin accumulation." Chemicals placed in this category have a nongenotoxic mechanism that has no relevance for extrapolation to renal tumors in humans. However, data on butyl alcohol exposure in drinking water to female rats demonstrate a dose-related increase in the severity of chronic progressive nephropathy, and an increased incidence of thyroid gland follicular cell hyperplasia and adenomas in mice. This review was focused towards oral exposures and did not address inhalation exposure of butyl alcohol.

BUTYL BUTYRYL LACTATE (BUTOXY-1-METHYL-2-OXOETHYL ESTER BUTANOIC ACID, 2-)
CAS: 7492-70-8

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

N-BUTYL ISOVALERATE
CAS: 109-19-3

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

3-BUTYLIDENEPHTHALIDE
CAS: 551-08-6

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

BUTYRIC ACID
CAS: 107-92-6
SEE HIGH MUL'S INGREDIENTS

CAPRYLIC/CAPRIC TRIGLYCERIDE
CAS: 65381-09-1
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

CARAMEL AND CARAMEL COLOR
CAS: 8028-89-5
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

CARBON
CAS: 7440-44-0
SEE MAJOR INGREDIENTS

CARBON DIOXIDE
CAS: 124-38-9

Number of relevant papers: 3

1. CO_2 induced acute respiratory acidosis and brain tissue intracellular pH: A SUP31P NMR study in swine -

Martoft L.1; Stødkilde-Jørgensen H.2; Forslid A.3; Pedersen H.D.1; Jørgensen P.F.1
Laboratory Animals, Volume 37, Number 3, 1 July 2003, pp. 241-248(8)

ABSTRACT: High concentration carbon dioxide (CO2) is used to promote pre-slaughter anaesthesia in swine and poultry, as well as short-lasting surgical anaesthesia and euthanasia in laboratory animals. Questions related to animal welfare have been raised, as CO2 anaesthesia does not set in momentarily. Carbon dioxide promotes anaesthesia by lowering the intracellular pH in the brain cells, but the dynamics of the changes in response to a high concentration of CO2 is not known. Based on 31P NMR spectroscopy, we describe CO2-induced changes in intracellular pH in the brains of . ve pigs inhaling 90% CO2 in ambient air for a period of 60 s, and compare the results to changes in arterial blood pH, PCO2, O2 saturation and HCO¡3 concentration. The intracellular pH paralleled the arterial pH and PCO2 during inhalation of CO2; and it is suggested that the acute reaction to CO2 inhalation mainly re□ ects respiratory acidosis, and not metabolic regulation as for example transmembrane □ uxes of H‡=HCO¡3 . The intracellular pH decreased to approximately 6.7 within the 60 s inhalation period, and the situation was metabolically reversible after the end of CO2 inhalation. The fast decrease in intracellular

pH supports the conclusion that high concentration CO2 leads to anaesthesia soon after the start of inhalation.

COMMENTS: The objective of this study was to assess the acute response of intracellular pH changes in brain of pigs induced by inhalation of 90% CO_2 in ambient air for a period of 60 seconds and to relate these changes to arterial blood. Intracellular pH decreased from the start of CO_2 inhalation period at a higher pace than that observed in arterial pH, and reached levels (6.7) lower than that observed in arterial pH. Reversal to pre-exposure conditions of intracellular pH was also rapid. The authors predict that the levels might have returned more slowly if the pigs had been allowed to respire freely due to CO_2 induced neuronal depression, which would slow the exhalation of CO_2. The objective of this work was to resolve questions related to animal welfare following the high concentrations of carbon dioxide used to promote pre-slaughter anaesthesia in livestock. Because of the high concentrations of CO_2 used in this study, the extrapolation to the effects of CO_2 exposure from cigarette smoke is difficult.

2. TOXICOLOGICAL EVALUATION OF HONEY AS AN INGREDIENT ADDED TO CIGARETTE TOBACCO

Mari S. Stavanja, Paul H. Ayres, Daniel R. Meckley, Betsy R. Bombick, Deborah H. Pence, Michael F. Borgerding, Michael J. Morton, Arnold T. Mosberg, James E. Swauger
Journal of Toxicology and Environmental Health, Part A, 66:1453–1473, 2003

ABSTRACT: A tiered testing strategy has been developed to evaluate the potential for new ingredients, tobacco processes, and technological developments to increase or reduce the biological activity that results from burning tobacco. In the manufacture of cigarettes, honey is used as a casing ingredient to impart both aroma and taste. The primary objective of this document is to summarize and interpret chemical and toxicological studies that have been conducted to evaluate the potential impact of honey on the biological activity of either mainstream cigarette smoke or cigarette smoke condensate. As part of ongoing stewardship efforts, cigarettes produced with honey (5% wet weight) as an alternative to invert sugar in tobacco casing material were subjected to extensive evaluation. Principal components of this evaluation were a determination of selected mainstream smoke constituent yields, Ames assay, sister chromatid exchange assay in Chinese hamster ovary cells, a 30-wk dermal tumor promotion evaluation of cigarette smoke condensate in SENCAR mice, and a 13-wk inhalation study of cigarette smoke in Sprague-Dawley rats. Comparative analytical evaluations demonstrated that the substitution of honey for invert sugar as a casing material in cigarettes had no significant impact on mainstream smoke chemistry. In addition, in vitro and in vivo studies demonstrated that cigarettes containing tobacco cased with honey had comparable biological activity to cigarettes containing invert sugar. Collectively, these data demonstrate that the use of honey as an alternative casing material in the manufactureof cigarettes does not alter the potential toxicity of cigarette smoke condensate (CSC) or cigarette smoke; therefore the use of honey as an ingredient added to cigarette tobacco is acceptable from a toxicological perspective.

COMMENTS: This paper compares the use of honey in place of invert sugar as casing material in cigarettes. No differences were observed in carbon dioxide measured in the mainstream smoke chemistry between the two cigarettes (mean = 41 - 42.2 mg/cig). No differences in toxicological endpoints were observed between the reference cigarette and those including honey. This paper has minor relevant to assessing the effects of carbon dioxide as an ingredient in cigarettes, but does conclude that the substitution of honey for invert sugar as a casing material does not significantly alter smoke chemistry.

3. Acute carbon dioxide exposure in healthy adults: evaluation of a novel means of investigating the stress response -

Kaye J.1; Buchanan F.2; Kendrick A.2; Johnson P.1; Lowry C.1; Bailey J.3; Nutt D.3; Lightman S.1 Source: Journal of Neuroendocrinology, Volume 16, Number 3, March 2004, pp. 256-264(9)

ABSTRACT: Acute hypercapnia was studied to assess its potential as a noninvasive and simple test for evoking neuroendocrine, cardiovascular and psychological responses to stress in man. A single breath of four concentrations of carbon dioxide, 5%, 25%, 35%, and 50% was administered to nine healthy volunteers in a randomized, single-blind fashion. Although no adverse effects occurred, most subjects were unable to take a full inspired vital capacity breath of 50%. In response to the remaining exposures, subjective and somatic symptoms of anxiety increased in a dose-dependent manner. Unlike 5% and 25% CO2, 35% stimulated significant adrenocorticotropic hormone and noradrenaline release at 2 min. and cortisol and prolactin release at 15 mins. following inhalation. This same dose also provoked a significant bradycardia that was followed by an acute pressor response. No significant habituation of psychological, hypothalamic-pitutary-adrenal (HPA)or cardiovascular responses following 35% CO2 was seen when this dose was repeated after 1 week. A single breath of 35% CO2 safety and reliably produced sympathetic and HPA axis activation and should prove a useful addition to currently available laboratory tests of the human stress response.

COMMENTS: While the aim of this study was to evaluate the stress response to acute CO_2, the data does indicate that the response to hypercapria in normal individuals is dose-dependent and anxiety produced is transit. Exposure to 35% CO_2 stimulated the release of cortisol, adrenocorticotropic prolactin and noradrenaline hormone but not at concentrations of 5% or 25%. A single breath of 35% CO_2 also produced a marked systolic response that was preceded by a significant and persistent bradycardia. The lower doses did not have significant effect on cardiovascular parameters or catecholamine release.

CARDAMOM OLEORESIN, OIL, EXTRACT, SEED OIL, AND POWDER
CAS: 8000-66-6
CAS: 96507-91-4

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

CAROB BEAN GUM, ABSOLUTE AND EXTRACT
CAS: 9000-40-2
CAS: 84961-45-5

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

BETA-CAROTENE
CAS: 7235-40-7

Number of relevant papers: 8

1. Bacterial Mutagenicity testing of 49 food ingredients gives very few positive results.

PRIVAL M J,; SIMMON V F; MORTELMANS K E
GENETIC TOXICOLOGY BRANCH, FOOD DRUG ADMINISTRATION, 200 C STREET SW, WASHINGTON, DC 20204, USA USA
Mutation Research , Volume: 260 , Number: 4 , Page: 321-330 , 1991

ABSTRACT: 49 substances permitted for use in food in the United States were tested for mutagenicity in the Ames Salmonella typhimurium assay and in Escherichia coli strain WP2. Four of these substances caused increases in revertant counts in S. typhimurium. Two of these four (papain and pepsin) were found to contain histidine, and therefore the results of the tests on these two substances could not be taken as demonstrating mutagenicity. The other two substances causing increases in revertant counts (hydrogen peroxide and potassium nitrite) were mutagenic. The results on one chemical, .beta.-carotene, were evaluated as inconclusive or questionable. The remaining 44 substances were nonmutagenic in the test systems used. It is concluded that, for those generally physiologically innocuous chemicals tested, there are very few 'false positives' in the bacterial test systems used.

COMMENTS: The Salmonella Ames test and E coli mutagenicity assays were used to evaluate the mutagenicity of a number of food ingredients. ß-carotene did not give a significant and reproducible increase in mutant counts and thus ß-carotene is classified as questionable or inconclusive rather than a nonmutagen. ß-carotene is an insoluble chemical and the Ames test is not considered to be suitable for testing insoluble substances.

2. beta-Carotene exacerbates DNA oxidative damage and modifies p53-related pathways of cell proliferation and apoptosis in cultured cells exposed to tobacco smoke condensate

Palozza P, Serini S, Di Nicuolo F, Boninsegna A, Torsello A, Maggiano N, Ranelletti FO, Wolf FI, Calviello G, Cittadini A.
Carcinogenesis. 2004 Aug;25(8):1315-25. Epub 2004 Apr 8.

ABSTRACT: Human intervention trials have suggested that supplemental b-carotene resulted in more cancer in smokers, whereas it was protective in non-smokers. However, the mechanisms underlying these effects are still unknown. The aim of this study was to evaluate the effects of an association of cigarette smoke condensate (tar) and b-carotene on DNA oxidative damage and molecular pathways involved in cell cycle progression and apoptosis in cultured cells. In RAT-1 fibroblasts, tar caused increased levels of 8-hydroxyl-20-deoxyguanosine (8-OHdG) and this effect was enhanced by the concomitant presence of b-carotene (0.5--4.0 mM) in a dose- and time-dependent manner. In contrast, b-carotene alone did not significantly modify it. Fibroblasts treated with tar alone decreased their cell growth with respect to control cells through an arrest of cell cycle progression in the G0/G1 phase and an induction of apoptosis. These effects were accompanied by an increased expression of p53, p21 and Bax and by a decreased expression of cyclin D1. In contrast, fibroblasts treated with tar and b-carotene, after an initial arrest of cell growth at 12 h, re-entered in cell cycle and were unable to undergo apoptosis at 36 h. Concomitantly, their p53 expression, after an increase at 12 h, progressively returned at basal levels at 36 h by a mechanism independent of Mdm2. Such a decrease was followed by a decrease in p21 and Bax expression and by an increase in cyclin D1 expression. Moreover, the presence of the carotenoid remarkably enhanced cyclooxygenase-2 expression induced by tar. During tar treatment, a depletion of b-carotene was observed in fibroblasts. The effects of tar and b-carotene on 8-OHdG levels, cell growth and apoptosis were also observed in Mv1Lu lung, MCF-7 mammary, Hep-2 larynx and LS-174 colon cancer cells. This study supports the evidence for potential detrimental effects of an association between b-carotene and cigarette smoke condensate.

COMMENTS: This study explores a new mechanism for carcinogenic association between ß-carotene and cigarette smoke using cultured cells exposed to a combination of ß-carotene and tar. Together, ß-carotene and tar caused significant increases in oxidative DNA damage over either alone. These effects were both dose- and time- dependent and were observed over a range of ß-carotene concentrations from 0.75 - 4 µM, which corresponds to the concentrations in serum of subjects receiving supplements in clinical trials. Exposure to these substances together resulted in increased cell growth using RAT-1 fibroblasts and a clonogenic assay. Similar results were observed when tested with a variety of human tumor cell lines. The authors conclude that pro oxidant action of ß-carotene exacerbates DNA oxidative damage caused by cigarette smoke and induce changes in p53-related pathways. At low concentrations, ß-carotene increased DNA resistance to oxidative damage.

3. Effect of alpha-tocopherol and beta-carotene supplementation on coronary heart disease during the 6-year post-trial follow-up in the ATBC study. - 2004 -

Tornwall ME, Virtamo J, Korhonen PA, Virtanen MJ, Taylor PR, Albanes D, Huttunen JK.
Eur Heart J. 2004 Jul;25(13):1171-8.

ABSTRACT: Aims To evaluate the 6-year post-trial effects of a-tocopherol and b-carotene supplementation on coronary heart disease (CHD) in the a-tocopherol, b-carotene cancer prevention (ATBC) study. Methods and results 29 133 male smokers, aged 50–69 years were randomised to receive a-tocopherol 50 mg, or b-carotene 20 mg, or both, or placebo daily for 5–8 years. At the beginning of the post-trial follow-up, 23 144 men were still at risk for a first-ever major coronary event (MCE), and 1255 men with pre-trial history of myocardial infarction (MI) were at risk for MCE. Post-trial risk for MCE (n ¼ 2059) was 0.95 (95% confidence interval 0.87–1.04) among a-tocopherol recipients compared with non-recipients, and 1.14 (1.04–1.24) among b-carotene recipients compared with non-recipients. The risk for non-fatal MI (n ¼ 993) was 0.96 (0.85–1.09) and 1.16 (1.03–1.32), and for fatal CHD (n ¼ 1066) 0.94 (0.83–1.06) and 1.11 (0.99–1.25), respectively. Among men with pre-trial MI no effects were observed in post-trial risk of MCE (n ¼ 257). Conclusion b-Carotene seemed to increase the post-trial risk of first-ever non-fatal MI but there is no plausible mechanism to support it. Our findings do not advocate the use of a-tocopherol or b-carotene supplements in prevention of CHD among male smokers.

COMMENTS: Research continues to accumulate to attempt to uncover the underlying mechanism of action of ß-carotene toxicity. High doses have been shown to increase risk of lung cancer among smokers. ß-carotene has been suggested as a singlet oxygen quencher. These investigators report on post-trial effects of ß-carotene on major coronary events such as non-fatal MI and fatal CHD. These studies indicate that ß-carotene possibly increases the post-trial risk of first ever non-fatal myocardial infarction but they failed to suggest a possible mechanism to explain this effect.

4. The enigma of beta-carotene in carcinogenesis: What can be learned from animal studies. -

Robert M. Russell
The American Society for Nutritional Sciences J. Nutr. 134:262S-268S, January 2004

ABSTRACT: ß-carotene and other carotenoids have been thought to have anti-cancer activity, either because of antioxidant activity or because of their ability to be converted to vitamin A. Nevertheless, two large scale intervention studies in humans using high doses of ß-carotene found that B-carotene supplementation resulted in more lung cancer rather than less lung cancer among smoking and asbestos exposed populations. Studies conducted in the ferret have elucidated molecular mechanisms behind this observation, in that high-dose ß-carotene and smoke exposure in these animals leads to squamous metaplasia, a pre-cancerous lesion in the lung. High dose ß-carotene in the smoke exposed animals was found to give rise to a number of transient oxidative metabolites, which include P450 enzymes that result in the destruction of retinoic acid, and diminished retinoid signaling, and enhanced cell proliferation. In addition, eccentric cleavage ß-carotene metabolites facilitate the binding of smoke derived carcinogens to DNA. In other ferret studies low dose ß-carotene smoke exposure provided mild protection against squamous metaplasia. Thus, it appears that the explanation of the

apparent paradoxical effects of ß-carotene on lung cancer is related to dose. The metabolism and breakdown of natural products should be thoroughly investigated in animal models before embarking on large scale intervention trials, particularly when using unusually high doses that greatly exceed normal dietary levels.

COMMENTS: The study used ferrets as an animal model to assess the effects of ß-carotene in smoke-exposed animals. Localized proliferation of alveolar cells and alveolar macrophages with keratinized squamous epithelium was observed in animals given high dose ß-carotene (equivalent to 30 mg/d in humans), and the most severe responses (focal proliferation of alveolar cells, squamous metaplasia, and alveolar wall destruction) were observed in those exposed to both beta carotene and smoke. Cell proliferation was observed in both groups, but highest in the lung tissue of ferrets exposed to both ß-carotene and smoke. Retinoic acid levels were lower in both smoke-exposed and ß-carotene- treated groups as compared to controls. Using *in vitro* experiments, the authors demonstrated that lower ß-carotene levels in animals exposed to smoke were due to enhanced molecular breakdown. The authors propose a mechanism by which ß-carotene breakdown products might induce P450 enzyme activity resulting in the destruction of retinoic acid, and subsequent diminished retinoid signaling. The interference of this signaling pathway results in enhanced cell proliferation in ferret lung tissue. Oxidative products of ß-carotene also facilitate binding of benzo[a]pyrene metabolites to DNA. However, these effects appear to occur at high ß-carotene doses only, and not associated with low doses (equivalent to 6 mg in humans).

5. beta-Carotene: A cancer chemopreventive agent or a co-carcinogen?

Paolini M, Abdel-Rahman SZ, Sapone A, Pedulli GF, Perocco P, Cantelli-Forti G, Legator MS.
Mutat Res. 2003 Jun;543(3):195-200.

ABSTRACT: Evidence from both epidemiological and experimental observations have fueled the belief that the high consumption of fruits and vegetables rich in carotenoids may help prevent cancer and heart disease in humans. Because of its well-documented antioxidant and antigenotoxic properties, the carotenoid ß-carotene (ßCT) gained most of the attention in the early 1980s and became one of the most extensively studied cancer chemopreventive agents in population-based trials supported by the National Cancer Institute. However, the results of three randomized lung cancer chemoprevention trials on ßCT supplementation unexpectedly contradicted the large body of epidemiological evidence relating to the potential benefits of dietary carotenoids. Not only did ßct show no benefit, itwas associated with significant increases in lung cancer incidence, cardiovascular diseases, and total mortality. These findings aroused widespread scientific debate that is still ongoing. It also raised the suspicion that ßCT may even possess co-carcinogenic properties. In this review, we summarize the current data on the co-carcinogenic properties of ßCT that is attributed to its role in the induction of carcinogen metabolizing enzymes and the over-generation of oxidative stress. The data presented provide convincing evidence of the harmful properties of this compound if given alone to smokers, or to individuals exposed to environmental carcinogens, as a micronutrient

supplement. This has now been directly verified in a medium-term cancer transformation bioassay. In the context of public health policies, while the benefits of a diet rich in a variety of fruits and vegetables should continue to be emphasized, the data presented here point to the need for consideration of the possible detrimental effects of certain isolated dietary supplements, before mass cancer chemoprevention clinical trials are conducted on human subjects. This is especially important for genetically predisposed individuals who are environmentally or occupationally exposed to mutagens and carcinogens, such as those found in tobacco smoke and in industrial settings.

COMMENTS: This document provides a review of the literature related to the protective and carcinogenic actions of ß-carotene. Although ß-carotene is known to act as an antioxidant, it can also behave as a pro-oxidant at high oxygen pressure. The author described that ß-carotene itself does not exert cell transforming activity, but enhances the bioactivity and carcinogenicity of other compounds (i.e. benzo[a]pyrene) either through an induction of metabolizing enzymes (CYP) or generation of oxidative stress. These effects were observed at realistic concentrations observed in clinical trials using ß-carotene as a dietary supplement.

6. In vitro investigations into the interaction of beta-carotene with DNA: evidence for the role of carbon-centered free radicals -

Jos C. S. Kleinjans 1*, Marcel H. M. van Herwijnen 1, Jan M. S. van Maanen 1, Lou M. Maas 1, Theo M. C. M. de Kok 1, Harald J. J. Moonen 1, and Jacob J. Briedé 1
Carcinogenesis Advance Access

ABSTRACT: Supplementation by ß-carotene has unexpectedly appeared to increase lung cancer risk among smokers. In order to explain this, it has been suggested that at high serum levels of ß-carotene, prooxidant characteristics of ß-carotene may become manifest, yieldingreactive oxygen species (ROS) and inducing oxidative DNA damage. It has further been hypothesized that cigarette smoke carcinogens such as benzo(a)pyrene (B[a]P) and/or B[a]P metabolites, may directly react with ß-carotene; furthermore, □-carotene oxidation products may have a role in the bioactivation of B[a]P analogous to the peroxide-shunt pathway of cytochrome P-450 supported by cumene hydroperoxide. The aim of this study was to assess the effects of □-carotene on the formation of B[a]P-DNA adducts and oxidative DNA damage in vitro in isolated DNA, applying as metabolizing systems rat liver and lung metabolizing fractions, and lung metabolizing fractions from smoking and non-smoking humans. We established that □-carotene in the presence of various metabolizing systems was not able to induce oxidative DNA damage (8-oxo-dG), although ß-carotene is capable of generating ROS spontaneously in the absence of metabolizing fractions. Also, we could not find an effect of ß-carotene on DNA adduct formation induced by B[a]P upon metabolic activation. We could however provide evidence of the occurrence of a carbon-centered □-carotene radical which was found to be able to interact with B[a]P, and to intercalate with DNA.

COMMENTS: This study assessed the *in vitro* effects of β-carotene concentrations comparable with serum levels obtained during human intervention trials. No induction of oxidative DNA damage or benzo(a)pyrene-DNA adduct formation was associated with β-carotene exposure in the presence of various metabolizing systems. However, the authors suggest that a carbon-centered β-carotene radical may be capable of interacting with DNA and contribute to the mutagenic effects of DNA adducts formed by carcinogens. They conclude that a complex interaction including β-carotene cancer-promoting and anti-carcinogenic properties may exist *in vivo* and requires further research.

7. Neoplastic and antineoplastic effects of beta-carotene on colorectal adenoma recurrence: Results of a randomized trial. -

Baron JA, Cole BF, Mott L, Haile R, Grau M, Church TR, Beck GJ, Greenberg ER. Journal of the National Cancer Institute. Vol. 95, No. 10. May 21, 2003

ABSTRACT: In two large, randomized prevention trials, supplementation with ß-carotene increased the risk of lung cancer. Subjects in these studies were predominantly cigarette smokers, and the adverse effects were concentrated among those who also drank alcohol. Although ß-carotene supplementation appeared not to increase the risk of cancer generally, it is not clear if smoking and/or alcohol use alters the effect of ß-carotene on carcinogenesis at sites outside the lung. Methods: We studied the effect of ß-carotene supplementation on colorectal adenoma recurrence among subjects in a multicenter double-blind, placebo-controlled clinical trial of antioxidants for the prevention of colorectal adenomas. A total of 864 subjects who had had an adenoma removed and were polyp-free were randomly assigned (in a factorial design) to receive ß-carotene (25 mg or placebo) and/or vitamins C and E in combination (1000 mg and 400 mg, respectively, or placebo), and were followed with colonoscopy for adenoma recurrence 1 year and 4 years after the qualifying endoscopy. A total of 707 subjects had two followup examinations and provided smoking and alcohol use data.Adjusted multivariate risk ratios (RRs) and 95% confidence intervals (CIs) were used to assess the effects of ß-carotene on adenoma recurrence. Results: Among subjects who neither smoked cigarettes nor drank alcohol, ß-carotene was associated with a marked decrease in the risk of one or morerecurrent adenomas (RR = 0.56, 95% CI = 0.35 to 0.89), but ß-carotene supplementation conferred a modest increase in the risk of recurrence among those who smoked (RR = 1.36, 95% CI = 0.70 to 2.62) or drank (RR = 1.13, 95% CI = 0.89 to 1.43). For participants who smoked cigarettes and also drank more than one alcoholic drink per day, ß-carotene doubled the risk of adenoma recurrence (RR = 2.07, 95% CI = 1.39 to 3.08; P for difference from nonsmoker/nondrinker RR < .001). Conclusion: Alcohol intake and cigarettesmoking appear to modify the effect of ß-carotene supplementation on the risk of colorectal adenoma recurrence.

COMMENTS: Evidence indicates that cigarette smoking plays a role in carcinogenic effects seen with ß-carotene supplementation. However, the increase in lung cancer incidence was also associated with alcohol consumption, leading to the hypothesis that alcohol intake modifies the effect of ß-carotene to increase lung cancer risk. In this clinical trial, ß-carotene supplementation was beneficial (anti-neoplastic) in subjects who

did not smoke or drink but the proneoplastic risk increased (doubled) among those who smoke and drank alcohol. The authors suggest that smoking and use of alcohol modifies the effects of ß-carotene on the risk of colorectal cancers.

8. Exposing ferrets to cigarette smoke and a pharmacological dose of beta-carotene supplementation enhance in vitro retinoic acid catabolism in lungs via induction of cytochrome P450 enzymes. -

Liu C, Russell RM, Wang XD.
J Nutr. 2003 Jan;133(1):173-9.

ABSTRACT: In our previous studies, we found lower levels of retinoic acid (RA) in the lungs of ferrets exposed to cigarette smoke and/or a pharmacological dose of ß-carotene. To determine whether this is involved in excessive catabolism of RA via cytochrome P450 (CYP) induction, we carried out in vitro incubations of RA with the lungmicrosomal fractions of ferrets with or without CYP inhibitors and antibodies against CYP. The polar metabolites(4-oxo-RA and 18-hydroxy-RA) of RA metabolism after the incubation were analyzed by HPLC. Expressions of CYP(1A1, 1A2, 2E1 and 3A1) were examined using Western blot analysis. Incubation of various concentrations of RA with the lung microsomal fraction from ferrets exposed to cigarette smoke, a pharmacological dose of ß-carotene or their combination dose-dependently increased the levels of 4-oxo-RA and 18-hydroxy-RA compared with that of the control ferrets. At all RA concentrations, this increase was the greatest in lung tissue from the combined treatment group. Furthermore, this enhanced RA catabolism was substantially (80%) inhibited by nonspecific CYP inhibitors (disulfiram and liarozole), but was partially (50%) inhibited by resveratrol (CYP1A1 inhibitor), -naphthoflavone (CYP1A2 inhibitor) and antibodies against CYP1A1 and CYP1A2. Cigarette smoke exposure and/or pharmacological doses of ß-carotene increased levels of CYP1A1 and 1A2 by three- to sixfold but not levels of 2E1 and 3A1 in ferret lung tissue. These findings suggest that low levels of RA in the lung of ferrets exposed tocigarette smoke and/or pharmacological doses of ß-carotene may be caused by the enhanced RA catabolism via induction of CYP, CYP1A1 and CYP1A2 in particular, which provides a possible explanation for enhanced lung carcinogenesis seen with pharmacological doses of ß-carotene supplementation in cigarette smokers.

COMMENTS: Earlier studies by this group reported that ferrets exposed to cigarette smoke and fed ß-carotene, had increased molecular markers of cellular proliferation and histopathological changes in lung tissue. This study examined induction of cytochrome p450 enzymes (CYP) in ferret lung by smoke exposure and pharmacological doses (equivalent to human dose of 30 mg/d) of β-carotene. CYP1A1 and CYP1A2 were markedly higher in lung tissue of ferrets exposed to smoke, β-carotene, or both as compared to controls. The authors also established links between CYP induction and retinoic acid catabolism by cigarettes and/or β-carotene. Because of the action of retinoic acid on blocking squamous metaplasia in bronchial epithelium, the authors suggest that reduced retinoic acid levels may contribute to lung carcinogenesis, in addition to the bioactivation of carcinogens due to induced cytochrome p450 enzymes.

CARROT OIL, SEED
CAS: 8015-88-1
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

4-CARVOMENTHENOL
CAS: 562-74-3
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

BETA-CARYOPHYLLENE
CAS: 87-44-5
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

BETA-CARYOPHYLLENE OXIDE
CAS: 1139-30-6
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

CASSIA BARK, BUDS, OILS, AND EXTRACT
CAS: 8007-80-5
CAS: 84961-46-6
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

CASTOREUM, LIQUID, EXTRACT, TINCTURE AND ABSOLUTE
CAS: 8023-83-4
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

CELERY SEED OIL
CAS: 89997-35-3
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

CELLULOSE AND CELLULOSE FIBER
CAS: 65996-61-4
CAS: 9004-34-6
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

CHAMOMILE FLOWER OIL, EXTRACT AND ABSOLUTE
CAS: 8002-66-2
CAS: 8015-92-7
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

CHICORY EXTRACT
CAS: 68650-43-1

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

CHOCOLATE AND CHOCOLATE LIQUOR

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

1,8-CINEOLE (EUCALYPTOL)
CAS: 470-82-6

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

CINNAMALDEHYDE
CAS: 104-55-2

Number of relevant papers: 2

1. Structure-Activity Relationships for the Mutagenicity and Carcinogenicity of Simple and alpha-beta Unsaturated Aldehydes - 2003 - EMBASE® - US$2.94

Benigni R, Passerini L, Rodomonte A.
Environ Mol Mutagen. 2003;42(3):136-43.

ABSTRACT: Aldehydes are important industrial compounds that are used for the synthesis of chemicals and pharmaceuticals and as solvents, food additives, and disinfectants. Because of their reactivity, aldehydes are able to interact with electron-rich biological macromolecules and adverse health effects have been reported, including general toxicity, allergenic reactions, mutagenicity, and carcinogenicity. The cost, time, and number of animals necessary to adequately screen these chemicals places serious limitations on the number of aldehydes whose health potential can be studied and points to the need of using alternative methods for assessing, at least in a preliminary way, the risks associated with the use of aldehydes. A method of choice is the study of quantitative structure–activity relationships (QSARs). In the present work, we present QSAR models for the mutagenicity and carcinogenicity of simple aldehydes and _-_ unsaturated aldehydes. The models point to the role of electrophilicity, bulkiness, and hydrophobicity in the genotoxic activity of the aldehydes and lend themselves to the prediction of the activity of other untested chemicals of the same class.

COMMENTS: Although cinnamaldehyde and citrao were found to be inactive in the NTP bioassay, there are several aldehydes that are suspected genotoxic carcinogens. These authors used QSAR analysis to determine toxicity of these two compounds based on molecular structure properties of these chemicals. Using their model, citral was described as extremely weak (well below the potency range of mutagens) and cinnamaldehyde was described as very weak.

2. Toxicology and carcinogenesis studies of microencapsulated trans-cinnamaldehyde in rats and mice -

Hooth MJ, Sills RC, Burka LT, Haseman JK, Witt KL, Orzech DP, Fuciarelli AF, Graves SW, Johnson JD, Bucher JR.
Food Chem Toxicol. 2004 Nov;42(11):1757-68.

ABSTRACT: trans-Cinnamaldehyde is a widely used natural ingredient that is added to foods and cosmetics as a flavoring and fragrance agent. Male and female F344/N rats and B6C3F1 mice were exposed to microencapsulated trans-cinnamaldehyde in the feed for three months or two years. All studies included untreated and vehicle control groups. In the three-month studies, rats and mice were given diets containing 4100, 8200, 16,500, or 33,000 ppm trans-cinnamaldehyde. In rats, feed consumption was reduced in all exposed groups. In mice, feed consumption was reduced in the highest dose groups. Body weights of all treated males were less than controls. Body weights were reduced in female rats exposed to 16,500 or 33,000 ppm and female mice exposed to 8200 ppm or greater. All rats survived to the end of the study but some male mice in the highest dose groups died due to inanition from unpalatability of the dosed feed. The incidence of squamous epithelial hyperplasia of the forestomach was significantly increased in rats exposed to 8200 ppm or greater and female mice exposed to 33,000 ppm. In mice, the incidence of olfactory epithelial degeneration of the nasal cavity was significantly increased in males and females exposed to 16,500 ppm and females exposed to 33,000 ppm. In the two-year studies, rats and mice were exposed to 1000, 2100, or 4100 ppm trans-cinnamaldehyde. Body weights were reduced in mice exposed to 2100 ppm and in rats and mice exposed to 4100 ppm. In rats, hippuric acid excretion was dose proportional indicating that absorption, metabolism, and excretion were not saturated. No neoplasms were attributed to trans-cinnamaldehyde in rats or mice. Squamous cell papillomas and carcinomas of the forestomach were observed in male and female mice but the incidences were within the NTP historical control range and were not considered to be related to trans-cinnamaldehyde exposure.

COMMENTS: Although the oral route of exposure was used in these studies, the results described are of interest. The authors selected to test and characterize the toxicity of microencapsulated trans-cinnamaldehyde because of its structural similarity to cinnamyl anthranilate and 3,4,5-trimethoxy-cinnamaldehyde, two known rodent carcinogens. In a 3-month study, both rats and mice were exposed to concentrations ranging from 4000 to 33,000 ppm. A 2 years study exposed the test animals to concentrations of 1000, 2100, 4100 ppm. As expected the forestomach was the target organ for both species. There was a significant increase in hyperplasia in both rats and mice and in mice, olfactory epithelial degeneration was reported of the nasal cavity. In the 2-year study, no neoplasms were observed but olfactory epithelial pigmentation was reported in mice.

CINNAMON BARK, BUDS, LEAF, OIL, AND EXTRACT
CAS: 8015-91-6
CAS: 8007-80-5

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

CINNAMYL ACETATE
CAS: 103-54-8

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

CINNAMYL ALCOHOL
CAS: 104-54-1

Number of relevant papers: 2

1. Toxicology databases and the concept of thresholds of toxicological concern as used by the JECFA for the safety evaluation of flavouring agents

Renwick AG.
Toxicol Lett. 2004 Apr 1;149(1-3):223-34.

ABSTRACT: Since 1996 the FAO/WHO Joint Expert Committee on Food Additives (JECFA) has evaluated the safety of 1259 flavouring substances, based on a decision tree that incorporates a series of thresholds of toxicological concern. Safety conclusions are based on the predicted consequences of metabolism and whether the estimated intake is above or below a threshold of toxicological concern that is relevant to that compound. Compounds are allocated to one of three structural classes, and the intake compared with a threshold of toxicological concern derived using data from chronic and sub-chronic toxicity studies on compounds in the same structural class. If the substance is predicted to be metabolised to innocuous products there is no safety concern if the intake is below the threshold, but suitable toxicity data on the compound or structural analogues are required if the intake exceeds the threshold. If the substance is not predicted to be metabolized to innocuous products, and the intake is below the appropriate threshold, safety evaluation is based on data on the compound or structural analogues. An additional threshold of 1.5 _g per day, derived from doses of investigated chemicals giving a calculated cancer risk of one in a million, is applied when appropriate toxicity data are not available.

COMMENTS: This paper addresses the concept of "threshold of toxicity" as it relates to safety assessments of flavoring agents. The decision-making process for safety evaluation is reviewed, including chemical structural class allocation, consideration of predicted metabolism, estimated intake (per capita) and a comparison of the intake with the threshold of toxicological concern. Substances structurally related to menthol were included in a summary of the application of the procedure and all 14 compounds were classified as "no safety concern". However, this assessment is directed towards additives in food and does not attempt to address inhalation exposures.

2. The FEMA GRAS assessment of cinnamyl derivatives used flavor ingredients

Adams, T.B., Cohen, S.M., Doull, J., Feron, V.J., Goodman, J.I., Marnett, L.J., Munro, I.C., Portoghese, P.S., Smith, R.L., Waddell, W.J., and Wagner, B.M. (2004) Food and Chemical Toxicology, 42, 157-185.

ABSTRACT: This publication is the seventh in a series of safety evaluations performed by the Expert Panel of the Flavor and Extract Manufacturers Association (FEMA). In 1993, the Panel initiated a comprehensive program to re-evaluate the safety of more than 1700 GRAS flavoring substances under conditions of intended use. Elements that are fundamental to the safety evaluation of flavor ingredients include exposure, structural analogy, metabolism, pharmacokinetics and toxicology. Flavor ingredients are evaluated individually and in the context of the available scientific information on the group of structurally related substances. Scientific data relevant to the safety evaluation of the use of cinnamyl derivatives as flavoring ingredients is evaluated.

COMMENT: This panel evaluated the safety of cinnamyl derivatives used as flavor ingredients. These compounds were reaffirmed as GRAS. Acute oral LD50 in mice and rats indicated a low level of toxicity. Reproductive/developmental studies with this compound indicted no observed effects. This panel did report that this compound was found to have inhibitory effects on platelet function. Increase inhibition of platelet aggregation correlated with increase lipophilicity of the test substance.

CINNAMYL CINNAMATE
CAS: 122-69-0

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

CITRAL
CAS: 5392-40-5

Number of relevant papers: 5

1. Structure-Activity Relationships for the Mutagenicity and Carcinogenicity of Simple and alpha-beta Unsaturated Aldehydes -

Benigni R, Passerini L, Rodomonte A.
Environ Mol Mutagen. 2003;42(3):136-43.

ABSTRACT: Aldehydes are important industrial compounds that are used for the synthesis of chemicals and pharmaceuticals and as solvents, food additives, and disinfectants. Because of their reactivity, aldehydes are able to interact with electron-rich biological macromolecules and adverse health effects have been reported, including general toxicity, allergenic reactions, mutagenicity, and carcinogenicity. The cost, time,

and number of animals necessary to adequately screen these chemicals places serious limitations on the number of aldehydes whose health potential can be studied and points to the need of using alternative methods for assessing, at least in a preliminary way, the risks associated with the use of aldehydes. A method of choice is the study of quantitative structure–activity relationships (QSARs). In the present work, we present QSAR models for the mutagenicity and carcinogenicity of simple aldehydes and _-_ unsaturated aldehydes. The models point to the role of electrophilicity, bulkiness, and hydrophobicity in the genotoxic activity of the aldehydes and lend themselves to the prediction of the activity of other untested chemicals of the same class.

COMMENTS: Although cinnamaldehyde and citral were found to be inactive in the NTP bioassay, there are several aldehydes that are suspected genotoxic carcinogens. These authors used QSAR analysis to determine toxicity of these two compounds based on molecular structure properties of these chemicals. Using their model, citral was described as extremely weak (well below the potency range of mutagens) and cinnamaldehyde was described as very weak.

2. Toxicology and carcinogenesis studies of microencapsulated Citral in rats and mice -

Ress NB, Hailey JR, Maronpot RR, Bucher JR, Travlos GS, Haserman JK, Orzech DP, Johnson JD, Hejmancik MR.
Toxicolgical Sciences. 71, 198-206, 2003

ABSTRACT: Citral, a widely used natural ingredient, is added to foods and cosmetics as a flavoring and fragrance agent. Male and female F344/N rats and B6C3F1 mice were exposed to microencapsulated citral in the feed for 14 weeks or two years. All studies included untreated and vehicle control groups. In the 14-week studies, rats and mice were given diets containing 3900, 7800, 15,600, or 31,300 ppm citral. In rats, food consumption was reduced in the two highest dose groups. In mice an apparent increase in food consumption was observed, but was due to mice scattering the feed. Body weights of all treated animals were less than controls. All rats and four male mice were killed moribund in the high dose groups. In rats, forestomach and kidney lesions were observed. At the higher doses, lesions observed in the bone marrow, testes, and thymus in rats and in the ovary in mice were considered related to inanition and resultant moribundity. In the two-year studies, rats were exposed to 1000, 2000, or 4000 ppm citral. Body weights were reduced in the 4000 ppm rats. Mice were exposed to 500, 1000, or 2000 ppm citral. Body weights in the 1000 and 2000 ppm groups were reduced. No neoplasms were attributed to citral in rats or mice. Malignant lymphoma occurred with a positive trend and was significantly greater than controls in female mice in the 2000 ppm group. However, the incidences were within the NTP historical control range and could not be clearly related to citral administration.

COMMENTS: Citral was administered through the diet of rats and mice and evaluated for toxicity and carcinogenicity. Exposures were conducted for 14 weeks and 2 years with maximum concentrations in the diet of 31,300 ppm and 4000 ppm, respectively. The

minimum daily doses in the 2-year study were more than 10 times greater than the average daily intake in humans. Palatability issues resulted in decreased food consumption and lower weight gain in both species. Transient treatment-related hematological and serum biochemical effects were noted in rats, but were consistent with physiological responses related to decreased food and water consumption. Nephropathy with renal tubule granular casts was observed in treated male rats from the 14-week treatment, but no citral-related kidney neoplasms were observed in the 2-year study. In mice, there was an increase in the incidences of malignant lymphoma in the highest treatment groups during the 2-year study, but this incidence was low and within the historical range of control female mice fed similar diets. Extrapolation of the findings of this study to the effects of citral as an ingredient in cigarettes is difficult because of the route of exposure (diet) and the high concentrations of citral used in this study which were far above the expected exposure through cigarette smoke.

3. Classification of Diverse Organic Compounds That Induce Chromosomal Aberrations in Chinese Hamster Cells -

McElroy NR, Thompson ED, Jurs PC.
J Chem Inf Comput Sci. 2003 Nov-Dec;43(6):2111-9.

ABSTRACT: A data set of 297 diverse organic compounds that cause varying degrees of chromosomal aberrations in Chinese hamster lung cells is examined. Responses of an assay are categorized as clastogenic (>10% aberrant cells) and nonclastogenic (<5% aberrant cells). Each of the compounds is represented by calculated structural descriptors that encode topological, geometric, electronic, and polar surface features. A genetic algorithm (GA) employing a k-nearest neighbor (kNN) fitness evaluator is used to iteratively search a reduced descriptor space to find small, information-rich subsets of descriptors that maximize the classification rates for clastogenic and nonclastogenic responses. To further improve modeling, a similarity measure using atom-pair descriptors is employed to create more homogeneous data subsets. Three different data sets are examined. Results for a set of 297 compounds using the GA-kNN method were 86.5% and 80.0% correct classification in the training set and prediction set, respectively. Results for a subset of 279 compounds in model 2 are 85.7% and 85.7% for the training and prediction sets, respectively. Results for a subset of 182 compounds in model 3 are 91.5% and 94.4% for the training and prediction sets, respectively. Creating smaller, more topologically similar data sets result in improved classification rates.

COMMENTS: Predictive classification models were designed that link molecular structure of 297 organic compounds to their genotoxic potential, as determined by chromosomal aberration assays using Chinese hamster lung cells. The predictive ability of the models was examined using external data sets. Citral was predicted correctly to be nonclastogenic, defined as inducing fewer than 5% aberrant cells. The relevance of this study to citral as an ingredient in cigarette smoke is minimal except for the potential of such predictive models to be applied to effects assessments for smoke components.

4. Analysis of thresholds for carcinogenicity. -

William J. Waddell ,
Toxicology Letters Volume 149, Issues 1-3 , 1 April 2004, Pages 415-419
Proceedings of EUROTOX 2003. The XLI European Congress of Toxicology. Science for Safety

ABSTACT: Re-evaluations of large prominent studies, e.g. the ED01 study and N-nitrosodiethylamine, unequivocally have demonstrated that thresholds exist for carcinogenicity when the dose–response curves for animal studies done at high doses are calculated according to fundamental principles of chemistry. This requires dose to be on a logarithmic scale and percent tumors on a linear scale. Fifteen compounds approved by the Flavor and Extract Manufacturers Association (FEMA) expert panel as Generally Recognized As Safe (GRAS) have been reported to be carcinogenic in rodent studies. The thresholds for tumors of these flavors were at least several orders of magnitude greater than the estimated daily dose of these flavoring agents to individuals in the United States. Similarly, comparisons of thresholds of carcinogenicity of chemicals and drugs to which humans are exposed with their exposure levels suggest that experimental animals are more sensitive to arcinogenicity than humans. The animal studies should be viewed as providing evidence for the safety of these flavors and other compounds at current levels of human exposure.

COMMENTS: This author has published extensively, presenting good evidence for thresholds of carcinogenicity of flavors. This paper examines the threshold for 6 compounds, providing estimates of the current level of exposure and a safety factor for each chemical. For citral the minimum safety ratio of 407 was suggested. The authors suggest that the actual safety ratios are probably greater.

5. Safety evaluations of food chemicals by "COMPACT" 1. A study of some acyclic terpenes

Lewis DF, Ioannides C, Walker R, Parke DV.
Food Chem Toxicol. 1994 Nov;32(11):1053-9.

ABSTRACT: A group of 19 acyclic terpenes have been evaluated for potential toxicity/carcinogenicity by molecular orbital determinations of their spatial and electronic parameters, and hence prediction of their metabolic activation or detoxication by the cytochrome P-450 (CYP) superfamily of mixed-function oxidase enzymes. Previous studies have characterized the spatial dimensions of the CYP1A1, 1A2 and 2E1 enzymes, which are known to activate mutagens and carcinogens and to be involved in other mechanisms of toxicity. None of the terpenes was found to have shape or electronic parameters appropriate for metabolic activation by CYP1A1 or 1A2, and hence they are unlikely to be carcinogenic or mutagenic. Furthermore, none of these chemicals had spatial parameters critical for substrates of CYP2E, and they are therefore unlikely to induce the formation of reactive oxygen species (ROS) or to initiate or promote malignancy or toxicity by mechanisms involving ROS. However, citral, and others of

these terpenes are known to undergo metabolism to carboxylic acids that may induce CYP4, and are therefore possible inducers of hepatic peroxisomal proliferation at high dosage, which may have implications for possible hepatotoxicity.

COMMENTS: Abstract sufficient, no additional comments needed.

CITRIC ACID
CAS: 77-92-9

Number of relevant papers: 1

1. Cough reflex induced by microinjection of citric acid into the larynx of guinea pigs: New coughing model. -

Tanaka M, Maruyama K.
J Pharmacol Sci. 2003 Dec;93(4):465-70.

ABSTRACT: We developed a new coughing model that evoked coughs by microinjection of citric acid into the larynx in unanesthetized unrestrained guinea pigs; additionally, we recorded synchronous sounds and waveforms of coughing utilizing built-in microphones and a whole body plethysmograph. The coughing model was able to distinguish a coughing response from other expiratory responses, such as an expiratory reflex or a sigh, by examining the waveform of the expiratory response and the existence of sound. It was not necessary to distinguish a cough from a sneeze, since the administration site was restricted to the larynx. Microinjection of 0.4 M citric acid, total of 20 _l (10 times, 2 _l at 30-s intervals), induced coughs (27.03 _ 4.03 coughs in 10-min observation) that were stable and independent of the inhalation volume. In the inhalation studies, animals were exposed to citric acid only once, because the number of coughs remarkably decreased with repeated administration at intervals of 24 h (tachyphylaxis). However our coughing model was able to repeatedly challenge the microinjection of citric acid at an interval of 24 h. These results indicated that this coughing model was highly sensitive and correctly assessed the cough response.

COMMENTS: Using unanesthetized, unrestrained guinea pigs, these authors demonstrated that microinjection of citric acid stimulated both the larynx and the bifurcation of the trachea, inducing cough and bronchoconstriction.

CITRONELLA OIL
CAS: 8000-29-1
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

CITRONELLOL
CAS: 106-22-9
SEE NEW INGREDIENTS

CLARY SAGE OIL AND EXTRACT
CAS: 8016-63-5
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

COCOA, COCOA SHELLS, EXTRACT, DISTILLATE, POWDER, ALKALIZED, ABSOLUTE AND TINCTURE
CAS: 8002-31-1
CAS: 84649-99-0
CAS: 68916-17-6
CAS: 95009-22-6
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

COCONUT OIL
CAS: 8001-31-8
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

COFFEE AND COFFEE SOLID EXTRACT
CAS: 8001-67-0
CAS: 68916-18-7
CAS: 84650-00-0
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

COGNAC WHITE AND GREEN OIL
CAS: 8016-21-5
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

CORIANDER EXTRACT, SEED, AND OIL
CAS: 8008-52-4
CAS: 84775-50-8
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

CORN STARCH
CAS: 9005-25-8
SEE NEW INGREDIENTS

BETA-DAMASCONE
CAS: 23726-92-3
CAS: 23726-91-2
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

DAVANA OIL
CAS: 8016-03-3
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

DECANAL
CAS: 112-31-2
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

DELTA-DECALACTONE
CAS: 705-86-2
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

GAMMA-DECALACTONE
CAS: 706-14-9
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

DECANOIC ACID
CAS: 334-48-5
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

DIACETYL
CAS: 431-03-8
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

DIETHYL MALONATE
CAS: 105-53-3
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

2,3-DIETHYLPYRAZINE
CAS: 15707-24-1
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

2,6-DIMETHOXYPHENOL
CAS: 91-10-1
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

DIMETHYL BENZYL CARBINYL BUTYRATE (ALPHA, ALPHA-DIMETHYLPHENETHYL BUTYRATE)
CAS: 10094-34-5
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

DIMETHYL SULFIDE
CAS: 18-50-9
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

3,4-DIMETHYL-1,2-CYCLOPENTADIONE
CAS: 13494-06-9
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

3,7-DIMETHYL-1,3,6-OCTATRIENE
CAS: 13877-91-3
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

4,5-DIMETHYL-3-HYDROXY-2,5-DIHYDROFURAN-2-ONE (3-HYDROXY-4,5-DIMETHYL-2(5H)FURANONE)
CAS: 28664-35-9
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

2, 5-DIMETHYL-4-HYDROXY-3(2H)-FURANONE (4-HYDROXY-2,5-DIMETHYL-3(2H)FURANONE) 3658-77-3
and
2,3-DIMETHYLPYRAZINE 5910-89-4
STANDARD

Number of relevant papers: 1

1. The influence of cigarette moisture to the chemistry of particulate phase smoke of a common commercial cigarette –
Zha, Q; Moldoveanu S C, (Reprint)
Beitraege zur Tabakforschung International , Volume: 21 , Number: 3 , Page: 184-191, October 2004 , 2004

ABSTRACT: This study presents the results on the influence of cigarette moisture content to the chemical composition of particulate phase smoke. Seventy-five selected compounds were monitored for the comparison of particulate phase smoke of a commercial full-flavored (FF) cigarette with three different moisture contents at 7.8%, 14.5% and 20.4%, respectively. It was demonstrated that the smoke of a dry cigarette is richer in lower molecular mass compounds than a regular cigarette. On the other hand, the smoke of a moist cigarette is richer in higher molecular mass compounds than a regular cigarette. To maximize the influence of cigarette moisture to the chemical composition, a separate set of measurements were done using only the first three puffs of smoke. The accumulation of moisture in the tobacco column of a burning cigarette may influence the smoke composition, as generated during burning. The differences between dry, regular and moist cigarettes were more obvious for the first three puffs.

COMMENTS: While this is not a health effect study, the results are interesting. These investigators compared the chemical composition of cigarette smoke from cigarettes with three moisture levels (dry-8.3%, regular-11.6%, and moist- 12.9%). The first three puffs showed the greatest differences. The nicotine content and total particulate matter (TPM) was reduced with increasing moisture. The data would indicate that the dry cigarette had higher percentage of semi-volatile compounds in TPM. The data presents additional evidence that the moisture content in cigarette significantly affects the chemistry of the particulate phase of smoke. Of the 75 compounds tested the more volatile compounds were more affected than the less volatile compounds. Compared to the first three puffs, the particulate phase of smoke from the entire cigarette was less sensitive to the moisture content.

3,7-DIMETHYL-6-OCTENOIC ACID (CITRONELLIC ACID)
CAS: 502-47-6
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ALPHA,PARA-DIMETHYLBENZYL ALCOHOL
CAS: 536-50-5
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

2,5-DIMETHYLPYRAZINE
CAS: 123-32-0
SEE HIGH MUL'S INGREDIENTS

DODECAHYDRO-3A,6,6,9A-TETRAMETHYLNAPHTHO (2,1-B)FURAN (1,5,5,9-TETRAMETHYL-13-OXATRICYCLO(8.3.0.0(4,9))TRIDECANE)
CAS: 3738-00-9
CAS: 6790-58-5
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

DELTA-DODECALACTONE
CAS: 713-95-1

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

GAMMA-DODECALACTONE
CAS: 2305-05-7

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL ACETATE
CAS: 141-78-6

Number of relevant papers: 1

1. Subchronic inhalation neurotoxicity studies of ethyl acetate in rats. -

Christoph GR, Hansen JF, Leung HW.
Neurotoxicology. 2003 Dec;24(6):861-74.

ABSTRACT: Rats were exposed to 0, 350, 750 or 1500 ppm of ethyl acetate by inhalation for 6 h per day, 5 days per week for 13 weeks. Functional observational battery (FOB) and motor activity tests occurred on non-exposure days during weeks 4, 8 and 13, after which tissues were microscopically examined for neuropathology. A subset of rats was monitored during a 4-week recovery period. Exposure to 750 and 1500 ppm, diminished behavioral responses to unexpected auditory stimuli during the exposure session and appeared to be an acute sedative effect. There were no signs of acute intoxication 30 min after exposure sessions ended. Rats exposed to 750 and 1500 ppm had reduced body weight, body weight gain, feed consumption, and feed efficiency, which fully or partially recovered within 4 weeks. Reductions in body weight gain and feed efficiency were observed in male rats exposed to 350 ppm. The principal behavioral effect of subchronic exposure was reduced motor activity in the 1500 ppm females, an effect that was not present after the 4-week recovery period. All other FOB and motor activity parameters were unaffected, and no pathology was observed in nervous system tissues. Operant sessions were conducted in another set of male rats preconditioned to a stable operant baseline under a multiple fixed ratio–fixed interval (FR–FI) schedule of food reinforcement. FR response rate, FR post-reinforcement pause duration, and the pattern of FI responding were not affected during or after the exposure series. In contrast, within-group FI rate for the treatment groups increased over time whereas those of the controls decreased. A historical control group, however, also showed a similar pattern of increase, indicating that these changes did not clearly represent a treatment related effect. Results from these studies indicate a LOEL of 350 ppm for systemic toxicity based on the decreased body weight gain in male rats, and a LOEL of 1500 ppm for neurotoxicity based on the transient reduction in motor activity in female rats. In conclusion, there was no evidence that subchronic exposure up to 1500 ppm ethyl acetate produced any enduring neurotoxic effects in rats.

COMMENTS: A large number of behavioral and neuropathological endpoints were measured by these investigators (37 functional observational battery tests, 2 motor activity and 5 operant tests). These studies suggest a LOEL of 350 ppm for decrease in body weight and a 1,500 ppm for reduction in motor activity. Even at this high concentration the authors reported no persistent adverse effect.

ETHYL ALCOHOL, INCLUDING SDA-4
CAS: 64-17-5
SEE MAJOR INGREDIENTS CATEGORY

ETHYL BENZOATE
CAS: 93-89-0
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL BUTYRATE
CAS: 105-54-4
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL CINNAMATE (PROPENIC ACID,3-PHENYL-,ETHYL ESTER,2-)
CAS: 103-36-6
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL DECANOATE
CAS: 110-38-3
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

4-ETHYL GUAIACOL (4-ETHYL-2-METHOXY-PHENOL)
CAS: 2785-89-9
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL HEPTANOATE
CAS: 106-30-9
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL HEXANOATE (ETHYL CAPROATE)
CAS: 123-66-0
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL ISOVALERATE
CAS: 108-64-5
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL LACTATE
CAS: 97-64-3
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL LAURATE
CAS: 106-33-2
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL LEVULINATE
CAS: 539-88-8
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL MALTOL
CAS: 4940-11-8
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL 2-METHYLBUTYRATE
CAS: 7452-79-1
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL METHYL PHENYLGLYCIDATE
CAS: 77-83-8
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL MYRISTATE
CAS: 124-06-1
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL NONANOATE
CAS: 123-29-5
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL OCTADECANOATE
CAS: 111-61-5
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL OCTANOATE
CAS: 106-32-1
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL OLEATE
CAS: 111-62-6
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

INGREDIENTS USED IN MIXTURE STUDIES TOBACCO SMOKE STUDIES

GENERAL COMMENTS:
In addition to studies that looked at individual ingredients, there were a few studies specifically designed to evaluate the potential effects of a large number of ingredients commonly added to cigarettes. The studies were unique in that all ingredients were tested by adding them in groups to a single research cigarette. The strength of this research is that these studies were specifically designed to determine (1) the effect of pyrolysis on the toxicity of the ingredients, (2) if new toxic substances were produced, (3) if the mixture of ingredients acts in a synergistic manner that might increase the toxicity of the inhaled smoke and (4) if these substances produced any identifiable new target organ toxicity not associated with cigarette smoke from cigarettes without the added ingredients. Well-established *in vitro* tests were included to identify mutagenicity (Ames test), cytotoxicity (neutral red uptake assay), carcinogenicity (two-stage mouse dermal assay), as well as a 90-day rat inhalation study. To complement these studies the chemical composition of the mainstream smoke from cigarettes with and without the added ingredients were also determined. These studies indicated that the addition of these chemicals, even at exaggerated levels, did not increase the bacterial mutagenicity, cytotoxicity nor the pathological response to the inhaled cigarettes with ingredients as compared to control cigarettes. When the results were compared to reference cigarettes without ingredients, the tests would indicate that the presence of these ingredients did not alter the biological activity. This model system represents a realistic model capable of detecting potential interactions among ingredient pyrolysis products together with various constituents known to be present in cigarette smoke. A number of the ingredients being reviewed in this report were included in these mixture studies. A list of these ingredients can be found below.

These extensive reviews would indicate that no chemical nor biological evidence has been presented to support the claim that ingredients added to cigarettes modifies the chemistry or biology activity of inhaled tobacco smoke.

The following ingredients were tested as a mixture added to cigarettes. Relevant mixture and review papers are listed below:

CITRONELLOL	**CAS: 106-22-9**
PARA-TOLUALDEHYDE	**CAS: 104-87-0**
ETHYL HEPTANOATE	**CAS: 106-30-9**
ISOAMYL FORMATE	**CAS: 110-45-2**
HEXYL ACETATE	**CAS: 142-92-7**
PECTIN	**CAS: 9000-69-7**
CORN STARCH	**CAS: 9005-25-8**
L-MENTHONE	**CAS: 14073-97-3**
ACETIC ACID	**CAS: 64-19-7**
ENZALDEHYDE	**CAS: 100-52-7**
BUTRIC ACID	**CAS: 107-92-6**
BETA-CARYOPHLLENE OXIDE	**CAS: 1139-30-6**
GAMMA-DECALACTONE	**CAS: 706-14-9**
2,5-DECALACTONE	**CAS: 123-32-0**
ETHYL BUTYRATE	**CAS: 105-54-4**
ETHYL DECANOATE	**CAS: 110-38-3**
ETHYL HEXANOATE	**CAS: 123-66-0**
ETHYL ISOVALERATE	**CAS: 108-64-5**
ETHYL LACTATE	**CAS: 97-64-3**
ETHYL LAURATE	**CAS: 106-33-2**
ETHYL MYRISTATE	**CAS: 124-06-1**
ETHYL OCTANOATE	**CAS: 106-32-1**
ETHYL PHENYLACETATE	**CAS: 101-97-3**
5-ETHYL-3-HYDROXY-4METHYL-2(5H)-FURANONE	**CAS: 698-10-2**
ISOAMYL ACETATE	**CAS: 123-92-2**
ISOBUTYL CINNAMATE	**CAS: 122-67-8**
ISOBUTYL PHENYLACETATE	**CAS: 102-13-6**
ISOBUTYRIC ACID	**CAS: 79-31-2**
2-METHYLPYRAZINE	**CAS: 109-08-0**
GAMMA-OCTALACTONE	**CAS: 104-50-7**
2,3-PENTANEDIONE	**CAS: 600-14-7**
2-PHENETHYL ACETATE	**CAS: 103-45-7**
PHENYLACETALDEHYDE	**CAS: 122-78-1**
SODIUMBICARBONATE	**CAS: 144-55-8**
2,3,5,6-TETRAMETHYLPYRAZINE	**CAS: 1124-11-4**
TRIETHYL CITRATE 77-93-0	
4-(2,6,6-TRIMETHYLCYCLOHEX-1-ENY) BUT-2-4- ONE (BETA-DAMASCONE)	**CAS: 23726-91-2; 35044-68-9**
GLYCEROL	**CAS: 56-81-5**
INVERTED SUGAR	**CAS: 8013-17-0**
CELLULOSE AND CELLULOSE	

FIBER	**CAS: 65996-61-4; 9004-34-6**
PROPYLENE GLYCOL	**CAS: 57-55-6**
METHOL AND L-MENTHOL	**CAS: 89-78-1; 216-51-5**
ETHYL ALCOHOL, INCLUDING SDA-4	**CAS: 64-17-5**
CHOCHOLATE AND CHOCOLATE LIQUOR	**CAS: N/A**
LACTIC ACID	**CAS: 50-21-5; 598-82-3**
SORBITOL	**CAS: 50-70-4**
AMMONIUM HYDROXIDE	**CAS: 1336-21-6**
GLUCOSE/DEXTROSE	**CAS: 50-99-7; 492-62-6**
SODIUM CARBONATE	**CAS: 497-19-8**
ETHYL 2-METHYLBUTYRATE	**CAS: 7452-79-1**
VANILLIN	**CAS: 121-33-5**

Each of the papers listed below, except for papers 7, 8, 9, and 10, has been reviewed and evaluated in previous review documents and will not be repeated here. The new papers have extensive abstracts fully defining the goals and conclusions reached by the authors.

1. Evaluation of the potential effects of ingredients added to cigarettes. Part 1: Cigarette design, testing approach, and review of results.
Food and Chemical Toxicology. Volume 40, Issue 1, pp. 77-91, January, 2002
E.L. Carmines et al

2. Evaluation of the potential effects of ingredients added to cigarettes. Part 2: Chemical composition of mainstream smoke.
AUTHORS: K. Rustemeiera, R. Stabberta, H.-J. Haussmanna, E. Roemera, E.L. Carmines.
SOURCE: Food and Chemical Toxicology.Vol. 40, Issue 1, pp. 93-104,January, 2002

3. Evaluation of the potential effects of ingredients added to cigarettes. Part 3: In vitro genotoxicity and cytotoxicity.
AUTHORS: E. Roemera, F.J. Tewesa, T.J. Meisgena, D.J.Veltela, E.L. Carmines.
SOURCE. Food and Chemical Toxicology.Vol. 40, Issue 1, pp.105-111, January, 2002

4. Evaluation of the potential effects of ingredients added to cigarettes. Part 4: Subchronic inhalation toxicity.
AUTHORS: P.M. Vanscheeuwijcka,*, A. Teredesaib, P.M. Terpstraa, J. Verbeecka, P. Kuhlb, B. Gerstenbergb, S. Gebelb, E.L. Carmines.
PUBLICATION SOURCE: Food and Chemical Toxicology, Volume 40, Issue 1, pp. 113-131, January, 2002

5. Toxicologic evaluation of flavor ingredients added to cigarette tobacco: skin painting bioassay of cigarette smoke condensate in SENCAR mice.
AUTHORS:C. L. Gaworski, J. D. Hecka, M. B. Bennetta and M. L. Wenk.
PUBLICATION SOURCE. Toxicology.Volume 139, Issues 1-2, 29 November 1999, Pages 1-17

6. Subchronic inhalation studies of complex fragrance mixtures in rats and hamsters.
AUTHORS: Fukayama Mark Y(a); Easterday Otho D; Serafino Patricia A; Renskers Kevin J ; North-Root Helen; Schrankel Kenneth R
SOURCE: Toxicology Letters 111(1-2 p 175-187 Dec. 20, 1999

7. The pyrolysis of tobacco ingredients -

Baker, R.R.;Bishop, L.J.
Journal of Analytical and Applied Pyrolysis, Volume 71, Issue 1, 1 March 2004, Pages 223-311

ABSTRACT: Relationships between tobacco components and smoke products are complex and often difficult to unravel. Pyrolysis experiments have commonly been used to establish such relationships. However, unless they are performed under dynamic conditions that are relevant to those that occur during tobacco burning, results can be obtained which have little resemblance to those obtained during cigarette smoking. The relevance of pyrolysis experiments to the behaviour of tobacco ingredients in a burning cigarette is considered. Based on the temperature, heating rate, oxygen levels and gas flow conditions that occur inside the burning zone of a cigarette, together with a review of relevant pyrolysis and smoking experiments, a set of pyrolysis conditions has been developed that approximates those occurring in the pyrolysis region of the burning cigarette. The conditions include heating the sample at 30 °C s−1 from 300 to 900 °C under a flow of 9% oxygen in nitrogen. Experiments on the pyrolytic behaviour of eleven relatively volatile substances under these conditions give results that are in good agreement with results from thirteen published studies in which cigarettes incorporating labelled versions of the substances were smoked. Subsequently, 291 single-compound tobacco ingredients have been pyrolysed under this set of conditions, most of which are relatively volatile. This enables the behaviour of these ingredients in a burning cigarette to be estimated in terms of intact transfer to mainstream smoke versus pyrolytic decomposition. It is predicted that almost a third of the substances would transfer to mainstream smoke at least 99% intact, and almost two-thirds would transfer 95% intact. Where pyrolytic decomposition does occur, the products are listed together with an estimate of the levels in smoke that would arise from the ingredient.

8. The effect of tobacco ingredients on smoke chemistry. Part I: Flavourings and additives

Baker RR; da Silva JRP; Smith G
Food and Chemical Toxicology 42(Supplement S): S3-S37, 2004. (34 refs.)

ABSTRACT: The effects of 450 tobacco ingredients added to tobacco on the forty-four "Hoffmann analytes" in mainstream cigarette smoke have been determined. These analytes are believed by regulatory authorities in the USA and Canada to be relevant to smoking related diseases. They are based on lists published by D. Hoffmann and co-workers of the American Health Foundation in New York. The ingredients comprised 431 flavours, 1 flavour/solvent, 1 solvent, 7 preservatives, 5 binders, 2 humectants, 2 process aids and I filler. The cigarettes containing mixtures of the ingredients were smoked using the standard ISO smoking machine conditions. The levels of the "Hoffmann analytes" in the smoke from the test cigarettes containing the ingredient mixture were compared to those from control cigarettes without the ingredients. In practice, flavouring ingredients are typically added to tobacco that also contains casing ingredients and reconstituted tobacco materials. In order to keep the tobacco mixtures as authentic as possible, three comparisons have been made in this study. These are: (a) control cigarette containing a typical US blended, cased tobacco incorporating reconstituted tobacco versus test cigarettes that had flavouring ingredients added to this tobacco; (b) control cigarette containing tobacco only versus test cigarettes with the tobacco cased and incorporating flavourings; (c) control cigarette containing tobacco only versus test cigarette incorporating additives made in an experimental sheet material. The significances of differences between the test and control cigarettes were determined using both the variability of the data on the specific occasion of the measurement, and also taking into account the long-term variability of the analytical measurements over the one-year period in which analyses were determined in the present study. This long-term variability was determined by measuring the levels of the 44 "Hoffmann analytes" in a reference cigarette on many occasions over the one-year period of this study. The ingredients were added to the experimental cigarettes at or above the maximum levels used commercially by British American Tobacco. The effect of the ingredient mixtures on total particulate matter and carbon monoxide levels in smoke was not significantly different to the control in most cases, and was never more than 10% with any ingredient mixture. It was found that, in most cases, the mixtures of flavouring ingredients (generally added in parts per million levels) had no statistically significant effect on the analyte smoke yields relative to the control cigarette. Occasionally with some of the mixtures, both increases and decreases were observed for some smoke analyte levels relative to the control cigarette. These differences were generally up to about 15% with the mixtures containing flavouring ingredients. The significance of many of the differences was not present when the long-term variability of the analytical methodology was taken into account. For the test cigarettes with ingredient mixtures containing casing ingredients, there were again no significant changes in smoke analyte levels in most cases. Those changes that were observed are as follows. Decreases in smoke levels were observed with some ingredient mixtures for most of the tobacco specific nitrosamines (up

to 24%), NOx, most of the phenols (up to 34%), benzo[a]pyrene, and some of the aromatic amines and miscellaneous organic compounds on the "Hoffmann list". Increases were observed for some test cigarettes in smoke ammonia, HCN, formaldehyde and lead levels (up to 24%).The significance of the ammonia and lead increases was not present when the long-term variability of the analytical methodology was taken into account. The yields of some carbonyl compounds in smoke were increased in one comparison with an additives mixture containing cellulosic components; in particular, formaldehyde was increased by 68%. This was the largest single change seen in any smoke analyte level in this study. These carbonyls are produced from the pyrolysis of cellulosic and other polysaccharide materials, present in the additives mixture. With this test cigarette, all tobacco specific nitrosamines, phenols, semi-volatile bases, NO, and some aromatic amines and miscellaneous organic compounds on the "Hoffmann list" were decreased, by up to 22%. The significance of many of these differences remained even when the long-term variability of the analytical methodology was taken into account. The levels of all other "Hoffmann analytes" in the smoke were not significantly different to those of the control cigarette. With the exception of the determinations of "tar", nicotine and carbon monoxide, there are currently no internationally recognised standard methods for measurement of the other "Hoffmann analytes". Each laboratory uses its own methods and there are large laboratory-to-laboratory variations, as well as variations over time in a given laboratory. Therefore, it is important that in any comparison of smoke analytes amongst different cigarettes, all the analytes should be measured in the same laboratory and at the same time. This was the case in the present study and all the methods have been validated internally.

9. The effect of tobacco ingredients on smoke chemistry. Part II: Casing ingredients

Baker RR, Pereira da Silva JR, Smith G.
Food Chem Toxicol. 2004;42 Suppl:S39-52.

This is the second part of a study in which the effects of adding a range of ingredients to tobacco on the chemistry of cigarette mainstream smoke are assessed. The examination of smoke chemistry has concentrated on those constituents in smoke that regulatory authorities in the USA and Canada believe to be relevant to smoking-related diseases. In this part of the study the effects of 29 casing ingredients and three humectants have been assessed at the maximum levels typically used on cigarettes by British American Tobacco. This brings the total number of ingredients assessed in Parts I and II of this study to 482. The casing ingredients were added at levels of up to 68 mg on the cigarettes. Their effects on smoke constituents were generally larger than the effects of flavouring ingredients, which were added at parts per million levels. Many of the casing ingredient mixtures either had no statistically significant effect on the level of the analytes investigated in smoke relative to a control cigarette, or they produced decreases of up to 44% in some cases. Those analytes that were increased in smoke are highlighted in this paper. The largest increases were for formaldehyde levels, up to 26 microg (73%) in one case, observed from casing mixtures containing sugar. This is most likely due to the generation of formaldehyde by pyrolysis of sugars. Occasional small increases were also observed for other analytes. However, the statistical significance of many of these

increases was not present when the long-term variability of the analytical method was taken into account. The significance and possible reasons for the increases are discussed.

10. An overview of the effects of tobacco ingredients on smoke chemistry and toxicity -

Baker RR, Massey ED, Smith G.
Food Chem Toxicol. 2004;42 Suppl:S53-83.

ABSTRACT: This paper presents an overview of a series of studies designed to assess the influence of 482 tobacco ingredients on cigarette smoke chemistry and toxicity. The studies are: pyrolysis of the ingredients; influence of the ingredients on smoke constituents believed by regulatory authorities to be relevant to smoking-related diseases ("Hoffmann analytes"); influence of the ingredients on in vitro genotoxicity and cytotoxicity of smoke partiulate matter; and influence of the ingredients on the inhalation toxicity of smoke. The present paper brings the salient features of these studies together. A pyrolysis technique has been developed which, as far as practicably possible, mimics the combustion conditions inside a burning cigarette. The results from 291 single-substance ingredients indicate that almost a third would transfer out of the cigarette burning zone at least 99% intact (i.e. less than 1% pyrolysis), and almost two thirds would transfer at least 95% intact. Of the ingredients that underwent some degree of pyrolysis, a few "Hoffmann analytes" were detected amongst the pyrolysis products of 19 ingredients. Taking into account maximum use levels, their maximum pyrolysis levels were generally small and often insignificant compared to the levels typically present in smoke. Possible exceptions were acetaldehyde and benzene from the pyrolysis of malic acid. However, subsequent smoke chemistry studies indicated that the maximum levels predicted from pyrolysis of this involatile substance were overestimated, suggesting that malic acid does not undergo complete pyrolysis in the burning cigarette and/or generates acetaldehyde and benzene at similar rates to that of tobacco on a per weight basis. When added to tobacco, many of the ingredient mixtures produced no significant effect on the levels of many of the "Hoffmann analytes" in smoke, while some produced increases or decreases relative to the relevant control cigarettes. The study has concentrated on the increases. Many of the differences were found to be not significant when the long-term variability of the analytical methodology was taken into account. However, even taking this into account, the smoke formaldehyde levels in two of the test cigarettes were significantly increased relative to their controls, by up to 26 microg (73%). These increases are likely to be due to the pyrolysis of sugars, cellulose and other polysaccharide materials. The activity of smoke particulate matter from cigarettes containing tobacco ingredients has been determined with three in vitro bioassays, two for genotoxicity and one for cytotoxicity. These were the Ames test, the mammalian cell micronucleus assay, and the neutral red uptake cytotoxicity assay. Within the sensitivity and specificity of these bioassays, the specific activity of the cigarette smoke particulate matter was not changed by the addition of ingredients to the cigarette. Three 90-day sub-chronic inhalation studies have been undertaken and histopathological and histomorphometric assessments made within the respiratory tracts of animals exposed to smoke from cigarettes containing the various ingredient mixtures and their control

cigarettes. The response due to tobacco smoke exposure was not distinguishable between the test and control cigarettes, indicating that the presence of the ingredients had made no discernable differences to the type and severity of the treatment-related changes.

RELEVANT REVIEWS & INTERESTING PAPERS

1. Evaluation of certain food additives and contaminants -

Sixty-first report of the Joint FAO/WHO Expert Committee on Food Additives
WHO Technical Report Series 922, 2004 Geneva

GENERAL COMMENT: In this document, examples of additives that were reviewed include citric acid, 2 methylheptanoic, citral, citronellol and much more.

ABSTRACT: This report represents the conclusions of a Joint FAO/WHO Expert Committee convened to evaluate the safety of various food additives, with a view to recommending acceptable daily intakes (ADIs) and to prepare specifications for the identity and purity of food additives.The first part of the report contains a general discussion of the principles governing the toxicological evaluation of food additives (including flavouring agents) and contaminants,assessments of intake, and the establishment and revision of specifications for food additives. A summary follows of the Committee's evaluations of toxicological and intake data on various specific food additives (a-amylase from Bacillus lichenformis containing a genetically engineered a-amylase gene from B. licheniformis, annatto extracts, curcumin, diacetyl and fatty acid esters of glycerol, D-tagatose, laccase from Myceliophthora thermophila expressed in Aspergillus oryzae, mixed xylanase, b-glucanase enzyme preparation produced by a strain of Humicola insolens, neotame, polyvinyl alcohol,quillaia extracts and xylanase from Thermomyces lanuginosus expressed in Fusarium venenatum), flavouring agents, a nutritional source of iron (ferrous glycinate, processed with citric acid), a disinfectant for drinking-water (sodium dichloroisocyanurate) and contaminants (cadmium and methylmercury). Annexed to the report are tables summarizing the Committee's recommendations for ADIs of the food additives, recommendations on the flavouring agents considered, and tolerable intakes of the contaminants considered, changes in the status of specifications and further information requested or desired.

COMMENTS: This is a massive report that one needs to be aware of since this Committee had access to documents called Technical Data Sheets, which were prepared using new or existing food additives and which had not been published because the detailed information on manufacturing processes described therein could be commercially sensitive. These documents, however, also contain valuable information, which was not made public, on chemical and technological approaches.

The Committee recognized the need for a working definition of the term "flavouring agent" and recommended that such a definition be agreed at a future meeting. At its present meeting, the Committee noted that a range of regulatory definitions of "flavouring" and similar terms exist in different countries and concluded that any definition would need to be elaborated in an international forum, such as the Codex Alimentarius Commission. The Committee reiterated the criteria that need to be met for an individual flavouring agent to be evaluated by the existing Procedure for the Safety Evaluation of Flavouring Agents: • The substance should be chemically defined, such that at least 95% of the commercially used material consists either of the named chemical, or of the named chemical and identified secondary constituents. The substance is added to food for flavouring purposes, including the generation of active flavouring substances during storage or processing of the food. • There is a valid estimate of current exposure to the named substance and, if appropriate, its breakdown or reaction products. Some substances that have a use as flavouring agents may have been evaluated previously by the Committee in relation to other food additive functions. The use of such a substance, or its breakdown or reaction products, as a flavouring agent is included in the relevant, previously-established ADI.

2. Human functional neuroimaging in nicotine and tobacco research: Basics, background, and beyond - 2004 –

F. Joseph McClernon and David G. Gilbert
Nicotine & Tobacco Research Volume 6, Number 6 : 941 - 959

ABSTRACT: Modern functional neuroimaging techniques allow nicotine and tobacco researchers to investigate the neurobiological basis of addiction in humans. We introduce the methods and measures of the following neuroimaging techniques: Electroencephalography and event-related cortical potentials, positron emission tomography, and functional magnetic resonance imaging. We outline strengths and limitations across modalities and describe new and emerging technologies. We provide summaries of recent neuroimaging findings in the field of nicotine and tobacco research for neurochemistry, smoking and nicotine administration, craving and cue-reactivity, cognitive and affective information processing, and tobacco withdrawal. We address limitations of studies to date and identify opportunities for future research.

3. **Effect of exposure to secondhand smoke on markers of inflammation: the ATTICA study –**

Demosthenes B. Panagiotakos PhDa, , , Christos Pitsavos MD, PhDa, Christina Chrysohoou MD, PhDa, John Skoumas MDa, Constadina Masoura MDa, Pavlos Toutouzas MD, PhDa and Christodoulos Stefanadis MD, PhDa
The American Journal of Medicine Volume 116, Issue 3 , 1 February 2004, Pages 145-150

ABSTRACT: We sought to investigate the effect of secondhand smoke exposure on inflammatory markers related to cardiovascular disease. Methods. During 2001 to 2002,

we randomly selected a stratified (age-sex) sample of adults without clinical evidence of cardiovascular disease. Exposure to secondhand smoke (>30 minutes per day and ≥1 day per week) was recorded. Multivariate regression analysis was used to evaluate the effects of exposure to secondhand smoke on levels of C-reactive protein, fibrinogen, homocysteine, and oxidized low-density lipoprotein (LDL) cholesterol, and on white blood cell count. Results. One hundred and thirty-seven (38%) of the 357 men who had never smoked and 211 (33%) of the 638 never-smoking women reported current exposure to secondhand smoke. Compared with those who were not exposed to secondhand smoke, those exposed more than 3 days per week had higher white blood cell counts (by 600 cells per μL; P = 0.02), as well as higher levels of C-reactive protein (by 0.08 mg/dL; P = 0.03), homocysteine (by 0.4 μmol/L; P = 0.002), fibrinogen (by 5.2 mg/dL; P = 0.4), and oxidized LDL cholesterol (by 3.3 mg/dL; P = 0.03), after adjusting for several potential confounders. Conclusion: Our findings suggest another pathophysiological mechanism by which exposure to secondhand smoke is associated with the development of atherosclerosis.

4. **Influence of smoking and sinus on the prevalence and incidence of type 2 diabetes amongst men: the northern Sweden MONICA study**

M. Eliasson1,2, K. Asplund2, S. Nasic2 & B. Rodu3
Journal of Internal Medicine Volume 256 Issue 2 Page 101 - August 2004

ABSTRACT: To explore the effect of smoking and smokeless tobacco, 'snus', on the risk of type 2 diabetes. Design. Population-based cross-sectional and prospective follow-up study in northern Sweden. Subjects. A total of 3384 men, aged 25–74 years, who participated in the MONICA study in 1986, 1990, 1994 or 1999, 1170 of whom had an oral glucose tolerance test. In 1999, 1757 men from previous cohorts returned for re-examination. Main outcome measures. We compared the prevalence of type 2 diabetes or pathological glucose tolerance (PGT) amongst tobacco users to that of nonusers at entry into the study and at follow-up, using odds ratios. Results. Compared with never users, the ageadjusted risk of prevalent clinically diagnosed diabetes for ever smokers was 1.88 (CI 1.17–3.0) and for smokers 1.74 (0.94–3.2). Corresponding odds ratios for snus users were 1.34 (0.65–2.7) and 1.18 (0.48–2.9). We found no increased risk of prevalent PGT in snus users or smokers. Former smokers and snus users had an insignificantly increased risk for PGT. Compared with nonusers, the age-adjusted risk of developing clinically diagnosed diabetes during follow-up was 4.63 (1.37–16) in consistent exclusive smokers, 3.20 (1.16–8.8) in ex-smokers and no cases in consistent snus users. The risk of PGT during follow-up was not increased in consistent tobacco users but evident, although not statistically significant, in those who quit snus during the follow-up period, 1.85 (0.60–5.7). Adjustment for physical activity and alcohol consumption did not change the major findings. Conclusions. The risk of diabetes for snus users was not significantly increased. Smoking was associated with prevalent and incident cases of diabetes. Ex-tobacco users tended towards more PGT.

COMMENTS: This paper describes an epidemiological study comparing the effects of smoking and smokeless tobacco use on type 2 diabetes. The study confirmed previous

findings that smoking is a risk factor for type 2 diabetes, but did not find a similar association with the use of smokeless tobacco.

5. Chemically induced renal tubule tumors in the laboratory rat and mouse: Review of the NCI/NTP database and categorization of renal carcinogens based on mechanistic information

Edward Lock; Gordon Hard
Critical Reviews in Toxicology, Volume 34, Number 3, May-June 2004, pp. 211-299(89)

Abstract: The incidence of renal tubule carcinogenesis in male and female rats or mice with 69 chemicals from the 513 bioassays conducted to date by the NCI/NTP has been collated, the chemicals categorized, and the relationship between carcinogenesis and renal tubule hyperplasia and exacerbation of the spontaneous, age-related rodent disease chronic progressive nephropathy (CPN) examined. Where information on mechanism or mode of action exists, the chemicals have been categorized based on their ability to directly or indirectly interact with renal DNA, or on their activity via epigenetic pathways involving either direct or indirect cytotoxicity with regenerative hyperplasia, or exacerbation of CPN. Nine chemicals were identified as directly interacting with DNA, with six of these producing renal tubule tumors at high incidence in rats of both sexes, and in some cases also in mice. Ochratoxin A was the most potent compound in this group, producing a high tumor incidence at very low doses, often with metastasis. Three chemicals were discussed in the context of indirect DNA damage mediated by an oxidative free radical mechanism, one of these being from the NTP database. A third category included four chemicals that had the potential to cause DNA damage following conjugation with glutathione and subsequent enzymatic activation to a reactive species, usually a thiol-containing entity. Two chemicals were allocated into the category involving a direct cytotoxic action on the renal tubule followed by sustained compensatory cell proliferation, while nine were included in a group where the cell loss and sustained increase in renal tubule cell turnover were dependent on lysosomal accumulation of the male rat-specific protein, 2-globulin. In a sixth category, morphologic evidence on two chemicals indicated that the renal tumors were a consequence of exacerbated CPN. For the remaining chemicals, there were no pertinent data enabling assignment to a mechanistic category. Accordingly, these chemicals, acting through an as yet unknown mechanism, were grouped as either being associated with an enhancement of CPN mechanism, were grouped as either being associated with an enhancement of CPN (category 7, 16 chemicals), or not associated with enhanced CPN (category 8, 4 chemicals). A ninth category dealt with 11 chemicals that were regarded as producing increases in renal tubule tumors that did not reach statistical significance. A 10th category discussed 6 chemicals that induced renal tumors in mice but not in rats, plus 8 chemicals that produced a low incidence of renal tubule tumors in mice that did not reach statistical significance. As more mechanistic data are generated, some chemicals will inevitably be placed in different groups, particularly those from categories 7 and 8. A large number of chemicals in the series exacerbated CPN, but those in

category 7 especially may be candidates for inclusion in category 6 when further information is gleaned from the relevant NTP studies. Also, new data on specific chemicals will probably expand category 5 as cytotoxicity and cell regeneration are identified as obligatory steps in renal carcinogenesis in more cases. Additional confirmatory outcomes arising from this review are that metastases from renal tubule tumors, while encountered with chemicals causing DNA damage, are rare with those acting through an epigenetic pathway, with the exception being fumonisin B1; that male rats and mice are generally more susceptible than female rats and mice to chemical induction of renal tubule tumors; and that a background of atypical tubule hyperplasia is a useful indicator reflecting a chemically associated renal tubule tumor response. With respect to renal tubule tumors and human risk assessment, chemicals in categories 1 and 2, and possibly 3, would currently be judged by linear default methods; chemicals in category 4 (and probably some in category 3) as exhibiting a threshold of activity warranting the benchmark approach; and those in categories 5 and 6 as representing mechanisms that have no relevance for extrapolation to humans.

COMMENTS: This paper provides a review of 69 chemicals tested in the National Cancer Institute / National Toxicology Program (NCI/NTP) carcinogenicity bioassay database. The selected chemicals are those that have shown an association with renal tubule tumors in rat and/or mouse, and was focused on oral exposures.

6. Cigarette smoking exacerbates chronic alcohol-induced brain damage: A preliminary metabolite imaging study -

Durazzo TC, Gazdzinski S, Banys P, Meyerhoff DJ.
Alcohol Clin Exp Res. 2004 Dec;28(12):1849-60.

ABSTRACT: Cigarette smoking is common among alcohol-dependent individuals. Nevertheless, previous research has typically not accounted for the potential independent or compounding effects of cigarette smoking on alcohol-induced brain injury and neurocognition. METHODS: Twenty-four 1-week-abstinent recovering alcoholics (RAs; 14 smokers and 10 nonsmokers) in treatment and 26 light-drinking controls (7 smokers and 19 nonsmokers) were compared on measures of common brain metabolites in gray matter and white matter of the major lobes, basal ganglia, midbrain, and cerebellar vermis, obtained via multislice short-echo time proton magnetic resonance spectroscopic imaging. Smoking and nonsmoking RAs were also contrasted on measures of neurocognitive functioning, as well as laboratory markers of drinking severity and nutritional status. RESULTS: Chronic alcohol dependence, independent of smoking, was associated with lower concentrations of frontal N-acetylaspartate (NAA) and frontal choline-containing compounds, as well as lower parietal and thalamic choline. Smoking RAs had lower NAA concentrations in frontal white matter and midbrain and lower midbrain choline than nonsmoking RAs. A four-group analysis of covariance also demonstrated that chronic cigarette smoking was associated with lower midbrain NAA and choline and with lower vermian choline. In smoking RAs, heavier drinking was associated with heavier smoking, which correlated with numerous subcortical metabolite abnormalities. The 1-week-abstinent smoking and nonsmoking RAs did not differ

significantly on a brief neurocognitive battery. In smoking RAs, lower cerebellar vermis NAA was associated with poorer visuomotor scanning speed and incidental learning, and in nonsmoking RAs lower vermis NAA was related to poorer visuospatial learning and memory. CONCLUSIONS: These human in vivo proton magnetic resonance spectroscopic imaging findings indicate that chronic cigarette smoking exacerbates chronic alcohol-induced neuronal injury and cell membrane damage in the frontal lobes of RAs and has independent adverse effects on neuronal viability and cell membranes in the midbrain and on cell membranes of the cerebellar vermis. Higher smoking levels are associated with metabolite concentrations in select subcortical structures. Greater consideration of the potential effects of comorbid cigarette smoking on alcohol-induced brain damage and other diseases affecting the central nervous system is warranted.

7**. Alert for an epidemic of oral cancer due to use of the betel quid substitutes gutkha and pan masala: A review of agents and causative mechanisms**

Urmila Nair, Helmut Bartsch and Jagadeesan Nair
Mutagenesis vol. 19 no. 4 pp. 251-262, July 2004

ABSTRACT: In south-east Asia, Taiwan and Papua New Guinea, smoking, alcohol consumption and chewing of betel quid with or without tobacco or areca nut with or without tobacco are the predominant causes of oral cancer. In most areas, betel quid consists of a mixture of areca nut, slaked lime, catechu and several condiments according to taste, wrapped in a betel leaf. Almost all habitual chewers use tobacco with or without the betel quid. In the last few decades, small, attractive and inexpensive sachets of betel quid substitutes have become widely available. Aggressively advertised and marketed, often claimed to be safer products, they are consumed by the very young and old alike, particularly in India, but also among migrant populations from these areas world wide. The product is basically a ¯avoured and sweetened dry mixture of areca nut, catechu and slaked lime with tobacco (gutkha) or without tobacco (pan masala). These products have been strongly implicated in the recent increase in the incidence of oral submucous ®brosis, especially in the very young, even after a short period of use. This precancerous lesion, which has a high rate of malignant transformation, is extremely debilitating and has no known cure. The use of tobacco with lime, betel quid with tobacco, betel quid without tobacco and areca nut have been classi®ed as carcinogenic to humans. As gutkha and pan masala are mixtures of several of these ingredients, their carcinogenic affect can be surmised. We review evidence that strongly supports causative mechanisms for genotoxicity and carcinogenicity of these substitute products. Although some recent curbs have been put on the manufacture and sale of these products, urgent action is needed to permanently ban gutkha and pan masala, together with the other established oral cancer-causing tobacco products. Further, education to reduce or eliminate home-made preparations needs to be accelerated.

COMMENTS: Well-marketed and conveniently packaged commercial preparations containing chewing tobacco with various combinations of lime, betel quid and areca nut have popularized the use of these products in Asia. The authors summarize available

evidence of the carcinogenic potential of these mixtures, and suggest a ban on products such as gutkha and pan masala.

8. **Alcohol, acetaldehyde, and digestive tract cancer**

SALASPURO, M. Alcohol, acetaldehyde, and digestive tract cancer. In: Nutrition and alcohol, pp. 393-411. Boca Raton, CRC Press, 2004.
Book Chapter

ABSTRACT N/A

COMMENTS: This monograph reviews the health issues associated with use of alcohol and states that cancer risk is dose-dependent and alcohol and smoking is synergistic, producing a greater effect together than either alone. Moderate smoking without drinking and moderate drinking without smoking had a slight or negative effect on esophageal cancer risk. But simultaneous exposure to the same moderate amounts increased risk 12 to 19-fold in men and women respectively.

SAFETY DATA SHEET

1. Identification

Product identifier **Brown Invert Syrup**

Other means of identification Nuvert™

Synonyms

Recommended use Not available.

Recommended restrictions None known.

Manufacturer/Importer/Supplier/Distributor information

- **Company name** International Molasses Corporation, Ltd.
- **Address** 88 Market Street
  Saddle Brook, NJ 07663
- **Telephone**: 1-201-845-4420
- **E-mail** info@internationalmolasses.com
- **Emergency phone number** 1-201-368-8036

2. Hazard(s) identification

Physical hazards Not classified.

Health hazards Not classified.

OSHA defined hazards Not classified.

Label elements

- **Hazard symbol** None.
- **Signal word** None.
- **Hazard statement** The mixture does not meet the criteria for classification.
- **Precautionary statement**
  - **Prevention** Observe good industrial hygiene practices.
  - **Response** Wash hands after handling.
  - **Storage** Store away from incompatible materials.
  - **Disposal** Dispose of waste and residues in accordance with local authority requirements.

Hazard(s) not otherwise classified (HNOC) None known.

Supplemental information Not applicable.

3. Composition/information on ingredients

Mixtures

Chemical name	CAS number	%
Invert Sugar	8013-17-0	100

4. First-aid measures

Inhalation	Move to fresh air. Call a physician if symptoms develop or persist.
Skin contact	Wash off with soap and water. Get medical attention if irritation develops and persists.
Eye contact	Rinse with water. Get medical attention if irritation develops and persists.
Ingestion	Rinse mouth. Get medical attention if symptoms occur.
Most important symptoms/effects, acute and delayed	Direct contact with eyes may cause temporary irritation.
Indication of immediate medical attention and special treatment needed	Treat symptomatically.
General information	Ensure that medical personnel are aware of the material(s) involved, and take precautions to protect themselves.

5. Fire-fighting measures

Suitable extinguishing media	Water fog. Foam. Dry chemical powder. Carbon dioxide (CO2).
Unsuitable extinguishing Media	None known.
Specific hazards arising from the chemical	During fire, gases hazardous to health may be formed.
Special protective equipment and precautions for firefighters	Self-contained breathing apparatus and full protective clothing must be worn in case of fire.
Fire-fighting equipment/instructions	In the event of fire, cool tanks with water spray.
Specific methods	Cool containers exposed to flames with water until well after the fire is out.

6. Accidental release measures

Personal Precautions
Keep unnecessary personnel away. Wear appropriate personal protective equipment. Ensure adequate ventilation.

Spillage
Spillages should be cleared up immediately and the floor surface cleaned.
Spillages should be disposed of in accordance with local, state and federal regulations.

7. Handling and storage

Precautions for safe handling	Use with adequate ventilation. Wear appropriate personal protective equipment. Avoid direct contact with eyes.
Conditions for safe storage, including any incompatibilities	Keep container tightly closed. Store in a well-ventilated place. Store away from incompatible materials (see Section 10 of the SDS).

8. Exposure controls/personal protection

Occupational exposure limits	No exposure limits noted for ingredient(s).
Biological limit values	No biological exposure limits noted for the ingredient(s).
Appropriate engineering Controls	General ventilation normally adequate.
Individual protection measures, such as personal protective equipment	
Eye/face protection	If contact is likely, safety glasses with side shields are recommended.
Skin protection	
Hand protection	Wear suitable gloves.
Other	Wear suitable protective clothing.
Respiratory protection	In case of insufficient ventilation, wear suitable respiratory equipment.
Thermal hazards	Wear appropriate thermal protective clothing, when necessary.
General hygiene Considerations	Always observe good personal hygiene measures, such as washing after handling the material and before eating, drinking, and/or smoking. Routinely wash work clothing and protective equipment to remove contaminants.

9. Physical and chemical properties

Appearance	Viscous liquid.
Physical state	Liquid.
Form	Liquid.
Color	Dark Brown
Odor	Sweet, Characteristic Sucrose
Odor threshold	Not available.
pH	5.0-6.0
Melting point/freezing point	Not available.
Initial boiling point and boiling Range	Not available.
Flash point	Not available.
Evaporation rate	Not available.
Flammability (solid, gas)	Not available.
Upper/lower flammability or explosive limits	
Flammability limit - lower (%)	Not available.
Flammability limit - upper (%)	Not available.
Explosive limit - lower (%)	Not available.
Explosive limit - upper (%)	Not available.
Vapor pressure	Not available.
Vapor density	Not available.
Relative density	Not available.
Solubility(ies)	
Solubility (water)	Soluble
Partition coefficient (n-octanol/water)	Not available.

Auto-ignition temperature	Not available.
Decomposition temperature	Not available.
Viscosity	Not available.

10. Stability and reactivity

Reactivity	The product is stable and non-reactive under normal conditions of use, storage and transport.
Chemical stability	Material is stable under normal conditions.
Possibility of hazardous Reactions	No dangerous reaction known under conditions of normal use.
Conditions to avoid	Contact with incompatible materials.
Incompatible materials	Strong oxidizing agents.
Hazardous decomposition Products	No hazardous decomposition products are known.

11. Toxicological information

Information on likely routes of exposure

Ingestion	No adverse effects due to ingestion are expected.
Inhalation	No adverse effects due to inhalation are expected.
Skin contact	May cause skin irritation.
Eye contact	May cause eye irritation.

Symptoms related to the physical, chemical and toxicological characteristics	Irritant effects.

Information on toxicological effects

Acute toxicity	Occupational exposure to the substance or mixture may cause adverse effects.
Skin corrosion/irritation	Prolonged skin contact may cause temporary irritation.
Serious eye damage/eye Irritation	Direct contact with eyes may cause temporary irritation.

Respiratory or skin sensitization

Respiratory sensitization	No data available.
Skin sensitization	No data available.

Germ cell mutagenicity	No data available to indicate product or any components present at greater than 0.1% are mutagenic or genotoxic.
Carcinogenicity	This product is not considered to be a carcinogen by IARC, ACGIH, NTP, or OSHA.
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.

12. Ecological information (Non-Mandatory)

13. Disposal considerations (Non-Mandatory)

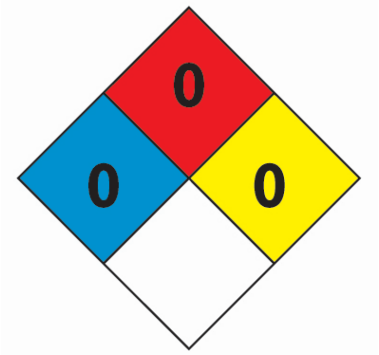
Waste Disposal Methods
Dispose of in compliance with local, state and federal laws and regulations.

14. Transport information (Non-Mandatory)

15. Regulatory information (Non-Mandatory)

16. Other information, including date of preparation or last revision

Issue date	04-June-2015
Revision date	21-January-2021
Version #	01
NFPA Ratings	



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