Toxicological profile for

Lovage extract, oil, root oil

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

Not applicable.

1.2. Synonyms

8016-31-7: FEMA No. 2649; FEMA No. 2650; FEMA No. 2651; Levisticum; Levisticum officinale oil; Lovage; Lovage (Levisticum officinale Koch); Lovage extract (Levisticum officinale Koch); Lovage herb oil; Lovage oil; Lovage roof oil; Lovage root oil; Lovage root oil (Levisticum officinale Koch); Lovage roots absolute; Oils, lovage; Oil of lovage; Smallage oil; Smellage oil; UNII-7534M4PQ6U; Lovage, oil (Levisticum officinale koch) (ChemIDplus)

84837-06-9: Levisticum; Levisticum officinale, ext.; Chinese lovage; EINECS 284-292-7 (ChemIDplus)

1.3. Molecular formula

Unspecified (CAS RN 8016-31-7) (ChemIDplus)

No data available to us at this time (CAS RN 84837-06-9).

1.4. Structural Formula

Not applicable.

1.5. Molecular weight (g/mol)

Not applicable.

1.6. CAS registration number

8016-31-7, 84837-06-9

1.7. Properties

1.7.1. Melting point

(°C): 21.47 (estimated) (CAS RN 8016-31-7) (EPISuite, 2017)

No data available to us at this time (CAS RN 84837-06-9).

1.7.2. Boiling point

(°C): 238.66 (estimated) (CAS RN 8016-31-7) (EPISuite, 2017)

No data available to us at this time (CAS RN 84837-06-9).

1.7.3. Solubility

18.97 mg/L at 25°C (estimated) (CAS RN 8016-31-7) (EPISuite, 2017)

No data available to us at this time (CAS RN 84837-06-9).

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

0.0497 mmHg at 25°C (estimated) (CAS RN 8016-31-7) (EPISuite, 2017)

No data available to us at this time (CAS RN 84837-06-9).

1.7.11. log Kow

3.96 (CAS RN 8016-31-7) (EPISuite, 2017)

No data available to us at this time (CAS RN 84837-06-9).

2. General information

2.1. Exposure

INCI Name	LEVISTICUM OFFICINALE OIL
Description	Levisticum Officinale Oil is the volatile oil distilled from the roots of the Lovage, Levisticum officinale, Apiaceae
CAS #	8016-31-7 / 84837-06-9
EC #	- / 284-292-7
Cosmetics Regulation provisions	
Functions	FRAGRANCE, TONIC

SCCS opinions	
Identified INGREDIENTS or substances e.g.	
INCI Name	LEVISTICUM OFFICINALE ROOT OIL
Description	Levisticum Officinale Root Oil is an essential oil obtained from the roots of the Lovage, Levisticum officinale, Apiaceae
CAS #	8016-31-7 / 84837-06-9
EC #	- / 284-292-7
Cosmetics Regulation provisions	
Functions	PERFUMING
SCCS opinions	
Identified INGREDIENTS or substances e.g.	

Additionally, Levisticum officinale leaf extract is used as a perfuming and skin conditioning agent, Levisticum officinale leaf oil as a perfuming agent and Levisticum officinale root extract as a fragrance, perfuming and skin conditioning agent in cosmetics in the EU (all CAS RN 84837-06-9).

As taken from CosIng (undated)

CAS RN 8016-31-7

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

Food category	Usual	Max	Food category	Usual	Max
Alcoholic beverages	28.55	32.21	Hard candy	0.02	0.02
Baked goods	5.14	7.07	Meat products	0.13	0.60
Chewing gum	8.00	8.00	Nonalcoholic beverages	4.79	6.01
Condiments, relishes	3.00	7.00	Soft candy	5.19	6.76
Frozen dairy	3.54	4.32	Soups	0.50	5.00
Gelatins, puddings	8.86	10.59	Sweet sauce	1.72	2.52
Gravies	1.00	2.00			

Estimated intake from flavouring use: 0.0001129 mg/kg bw/day.

As taken from Burdock, 2010.

Standard reference books state that the usual amounts of lovage preparations taken as herbal medicines or health supplements are 4-8 g/day of the powdered root or an equivalent quantity in an extract or 1-2 drops/day of the essential oil.

As taken from EFSA. 2009.

“Medicinal, Pharmaceutical, and Cosmetic: Lovage oil is used as a fragrance component in soaps, creams, lotions; and perfumes; with maximum use level of 0.2% reported in perfumes.

Food: Lovage oil and lovage extracts are used as flavor components in major food products, including alcoholic (liqueurs, etc.) and nonalcoholic beverages, frozen dairy desserts, candy, baked goods, gelatins and puddings, meat and meat products, and sweet sauces, among others. Average maximum use levels are generally below 0.005%, with the exceptions of 0.017% and about 0.013% (125 ppm) reported for lovage extract (type not indicated) in sweet sauces and in frozen dairy desserts, respectively.

Lovage (crude) is also reported used in alcoholic beverages, frozen dairy desserts, candy, and baked goods. Highest average maximum use level is 0.015% in alcoholic beverages.

Dietary Supplements/Health Foods: Root occasionally used in digestive formulations in capsules, tablets, and also as tea ingredient (FOSTER).

Traditional Medicine: Used as a diuretic, stomachic, expectorant, and emmenagogue. Conditions for which it is used include digestive problems, flatulence, gastric catarrh, skin problems, and menstrual difficulties. During China’s Cultural Revolution, the root was used as a substitute for wild-harvested danggui (Angelica sinensis), until cultivated supplies of the drug were developed (FOSTER AND YUE).”

As taken from Khan and Abourshed, 2010.

Lovage essential oil (no CAS RN given) is used as a flavour enhancer in non-medicinal natural health products, and Levisticum officinale and Levisticum officinale-ethanol. Decoctum (no CAS RNs given) are used in homeopathy (Health Canada, 2022).

2.2. Combustion products

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

Compound	Two stage heating		One stage heating	
	Abundance	Area%	Abundance	Area%
acetic acid	148053661	13.73	143609229	16.68
acetol	9899065	0.92	11885804	1.38
propylene glycol	251730178	23.34	208448050	24.21
furfural	46867170	4.35	38549587	4.48
furfuryl alcohol	25482802	2.36	21997332	2.56
5-methylfurfural	22645294	2.10	19606020	2.28

lactic acid	99065039	9.18	83390286	9.69
2,5-dimethyl-4-hydroxy-3(2H)-furanone	9230522	0.86	8719971	1.01
glycerol + 4,5-dimethylfurfural	28729809	2.66	17868796	2.08
2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	120680515	11.19	63197696	7.34
3,5-dihyroxy-2-methyl-4H-pyran-4-one	14522438	1.35	10543708	1.23
5-hydroxymethylfurfural	164132218	15.22	90475695	10.51
Total ion chromatogram	1078398279	100	860853425	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 CAS Number	**Max. cig appln. level (ppm)**	**Composition of pyrolysate (Compound, %)**	**Max. smoke (µg)**
Lovage oil (8016-31-7)	7.5	Butylphtalide 74.6 Butylidenephtalide 6.9 Isobutylidenephtalide 3.8 Sabinene+limonene 1.3 Valerophenone and/or phenylpentanone 0.7	3 0.3 0.1 0.05 0.03

2.3. Ingredient(s) from which it originates

“Lovage is an aromatic perennial herbaceous plant that grows wild in several areas in Europe. It is cultivated for extractive purposes in France, Germany, the Czech Republic, the Netherlands and the former Yugoslavia. It has large leaves with cylindrical stem, branched upper stalks, rhizome-type roots and yellow-greenish flowers arranged in dense clusters (July to August).”

Lovage oil - “The oil from the green parts is normally not manufactured and exhibits less interesting characteristics than the rhizome oil. Essential oil from rhizomes can be obtained by steam distillation of fresh or dried rhizomes with yields of 0.1 to 0.2%, or 0.6-1%, respectively.”

As taken from Burdock G.A (2010). Fenaroli’s Handbook of Flavor and Ingredients. Sixth Edition. CRC Press. ISBN 978-1-4200-9077-2.

“Lovage oil is obtained by steam distillation of the fresh [lovage] root”.

As taken from Khan and Abourashed, 2010

/Levisiticum/ A plant genus of the family APIACEAE (CAS RN 8016-31-7) (ChemIDplus).

Levisticum officinale leaf and root extracts are extracts of the leaves and roots, Levisticum officinale leaf oil is an essential oil obtained from the leaves (all CAS RN 84837-06-9) and Levisticum officinale oil and root oil (both CAS RNs 8016-31-7/84837-06-9) are the volatile and essential oils obtained from the roots of the Lovage, Levisticum officinale, Apiaceae.

As taken from CosIng (undated).

3. Status in legislation and other official guidance

Lovage oil (Levisticum officinale Koch) (CAS RN 8016-31-7) appears on the US FDA's list of Substances Added to Food (formerly EAFUS) as a flavoring agent or adjuvant, and is permitted under 21 CFR section 172.510 (natural flavoring substances and natural substances used in conjunction with flavors) (FDA, 2022a,b).

Lovage root oil and lovage leaf oil (both CAS RN 8016-31-7) are listed as fragrance ingredients by IFRA.

Oils, lovage (CAS RN 8016-31-7) are listed in the US EPA Toxic Substances Control Act (TSCA) and 2020 CDR TSCA Inv Active inventories.

The TSCA and 2020 CDR TSCA Inv Active inventories are available at https://sor.epa.gov/sor_internet/registry/substreg/LandingPage.do

EFSA Scientific Cooperation (ESCO, 2009) in 'Compendium of botanicals that have been reported to contain toxic, addictive, psychotropic, or other substances of concern' classifies the toxicity of substances present in lovage roots and recommends restrictions for use for: coumarin, furocoumarins (mainly bergapten, umbelliferone, psoralen); root seeds: imperatorin 12.82 mg/kg, 5-methoxypsoralen 6.38 mg/kg, psoralen 3.8 mg,kg, 8-methoxypsoralen 0.5 mg/kg.

As taken from EMA, 2012.

The CoE applied limits to the furocoumarin content of lovage extracts and essential oils (CoE, 2008).

Pre-registered under REACH ("envisaged registration deadline 31 May 2018" for oils, lovage (CAS RN 8016-31-7); "envisaged registration deadline 30 November 2010" for Levisticum officinale, ext. (CAS RN 84837-06-9)) (ECHA).

Neither CAS RN 8016-31-7 nor CAS RN 84837-06-9 are classified for packaging and labelling under Regulation (EC) No. 1272/2008 (ECHA, 2022).

Lovage oil (Levisticum officinale Koch) (CAS RN 8016-31-7) is listed in the US EPA InertFinder Database (2022) as approved for fragrance use pesticide products.

Lovage (FEMA no. 2649), lovage oil (EFMA no. 2651) and lovage extract (FEMA no. 2650) (Levisticum officinale Koch; all CAS RN 8016-31-7) have all been given GRAS (generally recognized as safe) status by FEMA (Hall and Oser, 1965).

According to New Zealand's Environmental Protection Authority, oils, lovage (CAS RN 8016-31-7) do not have an individual approval but may be used under an appropriate group standard (NZ EPA, 2006).

Levisticum officinale (no CAS RN given) is classified as a natural health product (NHP) under Schedule 1 item 1 (plant or plant material) of the NHP Regulations (Health Canada, 2022).

4. Metabolism/Pharmacokinetics

4.1. Metabolism/metabolites

No data available to us at this time.

4.2. Absorption, distribution and excretion

No data available to us at this time.

4.3. Interactions

No data available to us at this time.

5. Toxicity

5.1. Single dose toxicity

CAS RN 8016-31-7:

Organism	Test Type	Route	Reported Dose (Normalized Dose)	Effect	Source
mouse	LD50	oral	3400mg/kg (3400mg/kg)		Food and Cosmetics Toxicology. Vol. 16, Pg. 813, 1978.

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

“Tisserand and Balacs (1998) published a summary of data on the acute oral toxicity .... of lovage root oil. In their opinion, the oil is non-toxic and is safe to use unless there are other specific reasons: rodent oral LD50 values are in the range 2-5 g/kg, ...”

As taken from EMA, 2012.

5.2. Repeated dose toxicity

No data available to us at this time.

5.3. Reproduction toxicity

Groups of 10 female rats were administered lovage oil, by gavage, at 0, 100, 200 or 400 mg/kg bw/day from 7 days before mating and throughout mating and gestation until postnatal day 4. The rats were observed for clinical signs of toxicity, mortality and delivery of a litter, and offspring were weighed. Maternal toxicity (clinical signs of toxicity, decreased food consumption and/or decreased body weight gain) was noted at all dose levels. An increase in the number of still born pups and a decrease in pup viability was noted at 400 mg/kg bw/day, while pup body weight gain was decreased at 200 mg/kg bw/day and above. The NOAEL for maternal toxicity was <100 mg/kg bw/day while that for developmental toxicity was 100 mg/kg bw/day (Vollmuth et al. 1990).

5.4. Mutagenicity

No data available to us at this time.

5.5. Cytotoxicity

“.... We investigated the cytotoxic potential of essential oil from the leaves of a medicinal plant, Levisticum officinale (lovage) on head and neck squamous carcinoma cells (HNSCC). Cytotoxicity of lovage essential oil was investigated on the HNSCC cell line, UMSCC1. Additionally, we performed pharmacogenomics analyses.... Lovage essential oil extract had an IC50 value of 292.6 µg/ml. Genes involved in apoptosis, cancer, cellular growth and cell cycle regulation were the most prominently affected in microarray analyses. The three pathways to be most significantly regulated were extracellular signal-regulated kinase 5 (ERK5) signaling, integrin-linked kinase (ILK) signaling, virus entry via endocytic pathways and p53 signaling. Levisticum officinale essential oil inhibits human HNSCC cell growth” (Sertel et al., 2011).

“The aim of this study was to compare the antibacterial effects of several essential oils (EOs) alone and in combination against different Gram-positive and Gram-negative bacteria associated with

food products. Parsley, lovage, basil, and thyme EOs, as well as their mixtures (1:1, v/v), were tested against Bacillus cereus, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, and Salmonella typhimurium. The inhibitory effects ranged from strong (thyme EO against E. coli) to no inhibition (parsley EO against P. aeruginosa). Thyme EO exhibited strong (against E. coli), moderate (against S. typhimurium and B. cereus), or mild inhibitory effects (against P. aeruginosa and S. aureus), and basil EO showed mild (against E. coli and B. cereus) or no inhibitory effects (against S. typhimurium, P. aeruginosa, and S. aureus). Parsley and lovage EOs revealed no inhibitory effects against all tested strains. Combinations of lovage/thyme and basil/thyme EOs displayed antagonistic effects against all bacteria, parsley/thyme EOs against B. cereus, S. aureus, P. aeruginosa, and E. coli, and lovage/basil EOs against B. cereus and E. coli. Combinations of parsley/lovage and parsley/basil EOs exhibited indifferent effects against all bacteria. The combination of lovage/basil EO showed indifferent effect against S. aureus, P. aeruginosa, and S. typhimurium, and the combination parsley/thyme EO against S. typhimurium. Thyme EO has the highest percentage yield and antibacterial potential from all tested formulations; its combination with parsley, lovage, and basil EOs determines a reduction of its antibacterial activity. Hence, it is recommended to be used alone as the antibacterial agent." As taken from Semeniuc CA et al. 2017. J. Food Drug Anal. 25(2), 403-408. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28911683

"BACKGROUND AND OBJECTIVES: Essential oils are used for controlling and preventing human diseases and the application of those can often be quite safe and effective with no side effect. The essential oils have been found to have antiparasitic, antifungal, antiviral, antioxidant and especially antibacterial activity including antibacterial activity against tuberculosis. In this study the chemical composition and anti-TB activity of essential oil extracted fromLevisticum officinalehas been evaluated. MATERIALS AND METHODS: The essential oil of L. officinale was obtained by the hydro distillation method and the oil was analyzed by GC-FID and GC-MS techniques. The antibacterial activity of essential oil was evaluated through Minimum Inhibitory Concentration (MIC) assay using micro broth dilution method against multidrug-resistant Maycobacterium tuberculosis. The molecular modeling of major compounds was evaluated through molecular docking using Auto Dock Vina against-2-trans-enoyl-ACP reductase (InhA) as key enzyme in M. tuberclosis cell wall biosynthesis. RESULTS: The hydrodistillation on aerial parts of L. officinale yielded 2.5% v/w of essential oil. The major compounds of essential oil were identified as α-terpinenyl acetate (52.85%), β- phellandrene (10.26%) and neocnidilide (10.12%). The essentialoilshowed relatively good anti-MDRM. Tuberculosis with MIC = 252 µg/ml. The results of Molecular Docking showed that affinity of major compounds was comparable to isoniazid. CONCLUSION: The essential oil of aerial parts extracted from L. officinale was relatively active against MDRM. tuberculosis, and molecular docking showed the major compounds had high affinity to inhibit 2-trans-enoyl-acyl carrier protein reductase (InhA) as an important enzyme inM. Tuberculosis cell wall biosynthesis." As taken from Miran M et al. 2018. Iran. J. Microbiol. 10(6), 394-399. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/30873267

"Background: This study aimed to investigate Levisticum officinale hydroalcoholic extract (LOHE) effect on both cGMP signaling pathway and phosphodiesterase 5 (PDE5) gene expression pattern and to examine the role of LOHE in apoptosis induction of MCF-7 and MDA-MB-468 cell lines. Methods: The half maximal inhibitory concentration (IC50) of LOHE was examined in both cell lines using the MTT assay. Using IC50 values of LOHE on both cells, the type of cell death was detected by flowcytometric analysis. The values of PDE5 and cGMP were evaluated by real-time PCR and ELISA methods, respectively. Results: The IC50 values were measured as 150 µg/ml for MDA-MB-468 and 200 µg/ml for MCF-7. At 12 hour of treatment, a significant decrease in the PDE5 expression and maximum increase in the amount of intracellular cGMP were observed ($p < 0.05$). However, these effects were more noticeable in MDA-MB-468 triple-negative cells. Conclusion: Our data suggest that LOHE extract could be a potential source for new strategies towards targeting both PDE5 and cGMP signaling pathways." As taken from Lotfian Sargazi M et al. 2019. Iran. Biomed. J. 23(4), 280-286. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/30388886/

“Background: Studies have shown that zinc finger protein 703 (ZNF703) is overexpressed in breast cancer. Levisticum (L.) officinale is a herbal plant with proven medical characteristics in traditional medicine. The purpose of the present study was to evaluate the effect of hydroalcoholic extract of L. officinale (HELO) on both estrogen receptor-positive (ER+) and -negative (ER-) cell lines (MCF-7 and MDA-MB-468, respectively). Methods: The anti-proliferative and apoptotic activities of HELO were investigated on both cell lines using MTT and flow-cytometry methods. Real-time PCR was employed to determinate the changes in mRNA expression of the ZNF703 gene. Results: The 50% maximal inhibitory concentrations (IC50s) of HELO on ER+ and ER- cells were 200 and 150 µg/mL after 48 h-treatment. Statistically significant increases in both early and late apoptosis rates were seen in exposed cell lines. ZNF703 expression was less from 4 to 24 h HELO treatment than in untreated cells, and ZNF703 expression was higher in the more invasive MDA-MB-468 cells than in the less invasive MCF-7 cells. Our results demonstrated that HELO induces apoptosis and decreases cell growth in both cell lines. Conclusion: Our data suggest that HELO alters the mRNA levels of ZNF703 gene while inducing apoptotic cell death in breast cancer-derived cell lines. The use of ZNF703 suppression can be considered as a beneficial target in breast cancer research.” As taken from Mollashahee-Kohkan F et al. 2019. Rep. Biochem. Mol. Biol. 8(2), 119-125. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/31832434/

5.6. Carcinogenicity

No data available to us at this time.

5.7. Irritation/immunotoxicity

“A skin irritant. When heated to decomposition it emits acrid smoke and irritating fumes” (8016-31-7).

As taken from Lewis R (2008). Hazardous Chemicals: Desk Reference. 6th Edition.Wiley. ISBN 978-0-470-18024-4.

CAS RN 8016-31-7:

Type of Test	Route of Exposure or Administration	Species/Test System	Dose Data	Toxic Effects	Reference
Standard Draize test	Administration onto the skin	Rodent - rabbit	500 mg/24H	Moderate	FCTXAV Food and Cosmetics Toxicology. (London, UK) V.1-19, 1963-81. For publisher information, see FCTOD7. Volume(issue)/page/year: 16,813,1978
Standard Draize test	Administration onto the skin	Rodent – guinea pig	100%	Mild	FCTXAV Food and Cosmetics Toxicology. (London, UK) V.1-19, 1963-81. For publisher information, see FCTOD7. Volume(issue)/page/year: 16,813,1978

As taken from RTECS, 2019

“The extensive handling of lovage during harvest under prolonged exposure to strong sunlight induced dermatitis within a few hours with itching and erythema (Wolf 1995). After 36 hour on exposed arms and legs bullae and vesicles were formed with marked hyperpigmentation after 3 weeks…A similar case of dermatitis was described Vollman (1988) after contact with lovage oil.”

“Tisserand and Balacs (1998) published a summary of data on .... the skin irritation of lovage root oil. In their opinion, the oil is non-toxic and is safe to use unless there are other specific reasons: ..... causes a very mild irritation of the skin at >5 g/kg.”

As taken from EMA, 2012.

Lapeere et al (2013) reports the first case of contact dermatitis caused by lovage. According to the product data sheet provided by the manufacturer, the lovage essential oil used by the patient mainly contained beta-phellandrene, terpinyl acetate, cis-beta-ocimene, ligustilide and myrcene.

“Available data from one source indicate lovage root oil to be nonirritating and nonsensitizing to human skin, though one case of sensitization has been reported from another source.”

As taken from Khan and Abourshed, 2010.

"Nearly 80 essential oils (including 2 jasmine absolutes) have caused contact allergy. Fifty-five of these have been tested in consecutive patients suspected of contact dermatitis, and nine (laurel, turpentine, orange, tea tree, citronella, ylang-ylang, sandalwood, clove, and costus root) showed greater than 2% positive patch test reactions. Relevance data are generally missing or inadequate. Most reactions are caused by application of pure oils or high-concentration products. The clinical picture depends on the responsible product. Occupational contact dermatitis may occur in professionals performing massages. The (possible) allergens in essential oils are discussed. Several test allergens are available, but patients should preferably be tested with their own products. Co-reactivity with other essential oils and the fragrance mix is frequent, which may partly be explained by common ingredients. Patch test concentrations for essential oils are suggested." As taken from de Groot AC and Schmidt E. 2016. Dermatitis 27(4), 170-5. PubMed, 2017 available at: https://www.ncbi.nlm.nih.gov/pubmed/27427818

5.8. All other relevant types of toxicity

“Moderately toxic by ingestion.” (8016-31-7)

As taken from Lewis R (2008). Hazardous Chemicals: Desk Reference. 6th Edition.Wiley. ISBN 978-0-470-18024-4.

“The phototoxic effects of lovage oil on humans are not known.”

“Lovage extracts and oil have been reported to exhibit strong diuretic effects on rabbits and mice (LIST AND HO¨ RHAMMER); and also spasmolytic effects. Lovage root has recently been included in a review about herbals used for urinary tract problems.”

As taken from Khan and Abourashed, 2010.

“Lovage (Levisticum officinale W.D.J. Koch) is an aromatic plant from the Apiaceae (Umbelliferae) family used as a condiment in several regions of Europe and also described to have medicinal properties. While the aerial parts are used as foods, the roots are generally discarded. In the past, lovage roots were used in folk medicine for their diuretic, carminative, and spasmolytic properties. Therefore, the exploitation of this undervalued part of the plant can be a source of valuable bioactive compounds for food and/or pharmaceutical industries. Thus, in this study, extracts of different polarity were prepared and studied regarding their chemical composition and bioactive properties. To the best of our knowledge, the composition in phenolic compounds and the volatile profile of the n-hexane extract are reported for the first time. A total of 24 compounds were identified by GC-MS in the n-hexane extract, evidencing a high relative abundance of phthalides. A total of eight phenolic compounds were identified in lovage root extracts (decoction and hydroethanolic extract), with vanillic acid being the major compound. Regarding antioxidant activity, also reported for the first time, decoction and hydroethanolic extract exhibited a high antioxidant capacity in thiobarbituric acid reactive substances (TBARS) (179 ± 11 µg/mL) and in oxidative

hemolysis (OxHLIA) assays (510 ± 6 μg/mL), respectively. n-Hexane extract showed relevant anti-proliferative activity against all tumor cell lines tested (GI50, 48–69 μg/mL), despite inhibiting also the growth of a non-tumoral hepatocyte cell line, however, presenting a significantly higher GI50 value (147 μg/mL). This study revealed that lovage root, an agri-food residue, can be a source of valuable bioactive compounds also presenting biological properties that deserve being explored, which could lead to a circular economy for food and/or the pharmaceutical industry." As taken from Spréa RM et al. 2020. Resources 9(7), 81. Available at https://www.mdpi.com/2079-9276/9/7/81

"Levisticum officinale (Apiaceae) is a favorite food spice. Iranian folk medicine claims that it has a prominent antidyslipidemic property but this is not documented scientifically so far. This study evaluated antidyslipidemic and the other antidiabetic aspects of the stem and leaf hydroalcoholic extract of it (LOE). Regarding oral glucose tolerance test results, LOE (500 mg/kg) administration 30 min before glucose loading significantly decreased the blood glucose level (13%) at 90 min in male rats. Additionally, LOE treatment (500 mg/kg, orally, once a day) for 14 days significantly reduced the serum glucose level (24.97%) and markedly improved the lipid profile and the insulin, creatinine, alanine aminotransferase and aspartate aminotransferase serum levels in diabetic rats. Moreover, LOE effectively amended the impaired antioxidant status and ameliorated lipid peroxidation in the plasma and pancreas and liver tissues of diabetics. Also, 14 days LOE treatment, significantly decreased the renal sodium-glucose cotransporter 2 and facilitated glucose transporter 2 (GLUT2) mRNA levels and GLUT2 gene expression in the enterocytes of jejunum tissue in comparison with diabetic untreated rats. HPLC method revealed the presence of chlorogenic acid, rosmarinic acid, caffeic acid, quercetin and luteolin and GC-MS analysis detected bioactive compounds like phthalides, thymol, phytol, hexanoic acid, carene and menthofuran. LOE showed α-amylase (αA) inhibitory activity and in silico studies predicted that among extract ingredients luteolin, quercetin, rosmarinic, caffeic, and hexanoic acids have the greatest αA inhibition potecy. Thus, current results justify antidyslipidemic value of L. officinale and shed light on more antidiabetic health benefits of it." As taken from Ghaedi N et al. 2020. Iran. J. Pharm. Res. 19(1), 231-250. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/32922483/

Several plants have the potential to protect essential reproductive processes such as spermatogenesis or steroidogenesis, however, effective concentrations and main mechanisms of action are still unknown. This in vitro study was aimed to assess the effects of Apium graveolens L., Levisticum officinale, and Calendula officinalis L. extracts on the structural integrity, functional activity and gap junctional intercellular communication (GJIC) in mice Leydig cells. TM3 cells were grown in the presence of experimental extracts (37.5; 75; 150 and 300 μg/ml) for 24 h. For the present study, high-performance liquid chromatography analysis was used to quantify flavonoids or phenolic acids. Subsequently, Leydig cell viability was assessed by alamarBlue assay, while the cell membrane integrity was detected by 5-carboxyfluorescein diacetate-acetoxymethyl ester. The level of steroid hormones production was determined by enzyme-linked immunosorbent assay. Additionally, GJIC was assessed by scalpel loading/dye transfer assay. According to our results, Apium graveolens L. significantly increased the viability and cell membrane integrity at 75 μg/ml (109.0±4.3%) followed by a decline at 300 μg/ml (89.4±2.3%). In case of Levisticum officinale and Calendula officinalis L. was observed significant decrease at 150 μg/ml (88.8±11.66%; 87.4±6.0%) and 300 μg/ml (86.2±9.3%; 84.1±4.6%). Furthermore, Apium graveolens L. significantly increased the progesterone and testosterone production (75 and 150 μg/ml) however, Levisticum officinale and Calendula officinalis L. significantly reduced steroid hormones synthesis at 150 and 300 μg/ml. Finally, the disturbance of GJIC was significantly affected at 300 μg/ml of Levisticum officinale (82.5±7.7%) and Calendula officinalis L. (79.8±7.0%). The balanced concentration ratio may support the Leydig cell function, steroidogenesis as well as all essential parameters that may significantly improve reproductive functions.

As taken from Jambor et al. 2021.

6. Functional effects on

6.1. Broncho/pulmonary system

No data available to us at this time.

6.2. Cardiovascular system

“Herbs believed to contain coumarin or coumarin derivatives include… lovage root. These herbal products should be considered theoretical risks for prolonged bleeding at this point. Until more information becomes available, it would be wise to discourage use of any of these herbs in patients taking warfarin or who are undergoing any type of surgical procedure.”

As taken from Heyneman CA. Preoperative Considerations: Which Herbal Products Should Be Discontinued Before Surgery? Critical Care Nurse. 2003; 23: 116-124 available at http://ccn.aacnjournals.org/cgi/content/full/23/2/116

6.3. Nervous system

“Levisticum officinale (Apiaceae) has been identified as a medicinal plant in traditional medicine, with the anti-inflammatory, antioxidant, and anticholinesterase activities. The present study aims to evaluate the effects of Levisticum officinale extract (LOE) on lipopolysaccharide (LPS)-induced learning and memory deficits and to examine its potential mechanisms. LOE was administered to adult male Wistar rats at doses of 100, 200, and 400 mg kg-1 for a week. Later, LPS was intraperitoneally injected at a dose of 1 mg kg-1 to induce neuroinflammation, and treatment with LOE continued for 3 more weeks. Behavioral, biochemical, and molecular analyses were performed at the end of the experiment. Moreover, quantitative immunohistochemical assessments of the expression of Ki-67 (intracellular proliferation marker) in the hippocampus were performed. The results revealed that LPS injection caused spatial memory impairment in the rats. Daily LOE treatment at applied doses for 4 weeks attenuated spatial learning and memory deficits in LPS-injected rats. Furthermore, LPS significantly increased the mRNA expression level of interleukin-6 in the hippocampus, which was accompanied by decreased brain-derived neurotrophic factor (BDNF) mRNA expression levels. Moreover, LPS increased the levels of malondialdehyde, reduced the antioxidant enzyme activities of catalase and superoxide dismutase in the hippocampus, and impaired neurogenesis. However, pre-treatment with LOE at a dose of 100 mg kg-1 significantly reversed the LPS-induced changes, and improved neurogenesis. In conclusion, the beneficial effect of LOE on the improvement of learning and memory could be attributed to its anti-inflammatory and antioxidant activities, along with its ability to increase BDNF expression and neurogenesis in the hippocampus.” As taken from Amraie E et al. 2020. Food Funct. 11(7), 6608-6621. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/32648872/

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 Lovage oil and extract 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 Lovage oil and extract. Table below provides tested level(s) and specific endpoint(s).

Endpoint	Tested level (ppm)	Reference
Smoke chemistry	109 (8016-31-7)	Carmines, 2002 & Rustemeier et al., 2002
	12 (8016-31-7)	Baker et al., 2004a
	1,000 (No CAS) 2,000 (No CAS and 8016-31-7)	JTI KB Study Report(s)
	8,550	Gaworski et al., 2011 & Coggins et al., 2011b
	36	Roemer et al, 2014
In vitro genotoxicity	109 (8016-31-7)	Carmines, 2002 & Roemer et al., 2002
	12 (8016-31-7)	Baker et al., 2004c
	1,000 (8016-31-7)	Renne et al., 2006
	1,000 (No CAS) 2,000 (No CAS and 8016-31-7)	JTI KB Study Report(s)
	123 (8016-31-7)	fGLH Study Report (2010)
	8,550	Gaworski et al., 2011 & Coggins et al., 2011b
	36	Roemer et al, 2014
In vitro cytotoxicity	109 (8016-31-7)	Carmines, 2002 & Roemer et al., 2002
	12 (8016-31-7)	Baker et al., 2004c
	1,000 (No CAS) 2,000 (No CAS and 8016-31-7)	JTI KB Study Report(s)
	123 (8016-31-7)	fGLH Study Report (2010)
	8,550	Gaworski et al., 2011 & Coggins et al., 2011b
	36	Roemer et al, 2014
Inhalation study	0.7 (8016-31-7)	Gaworski et al., 1998
	109 (8016-31-7)	Carmines, 2002 & Vanscheeuwijck et al., 2002
	12 (8016-31-7)	Baker et al., 2004c
	1,000 (8016-31-7)	Renne et al., 2006
	1,000 (No CAS) 2,000 (No CAS and 8016-31-7)	JTI KB Study Report(s)
	36	Schramke et al, 2014
Skin painting	1 (8016-31-7)	Gaworski et al., 1999

	1,000 (No CAS) 2,000 (No CAS and 8016-31-7)	JTI KB Study Report(s)
In vivo genotoxicity	36	Schramke et al, 2014

9. Heated/vapor emissions toxicity

Total particulate matter (TPM) from heated (tobacco or nicotine) product(s) containing Lovage Root Oil (8016-31-7) was tested in a battery of in vitro and/or in vivo test(s). Within the sensitivity and specificity of the bioassay(s) the activity of the TPM was not increased by the addition of containing Lovage Root Oil (8016-31-7) when compared to TPM from 3R4F cigarettes. The table below provides tested level(s) and specific endpoint(s).

Endpoint	Tested level (ppm)	Reference
In vitro genotoxicity	19.5	JTI KB Study Report(s)
In vitro cytotoxicity	19.5	JTI KB Study Report(s)

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

Endpoint	Tested level (ppm)	Reference
Aerosol chemistry	8	Labstat International Inc. (2021)
In vitro genotoxicity	8	Labstat International Inc. (2022)
In vitro cytotoxicity	8	Labstat International Inc. (2022)

10. Ecotoxicity

10.1. Environmental fate

EPISuite provides the following information on CAS RN 8016-31-7:

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

Bond Method :	1.03E-003 atm-m3/mole (1.04E+002 Pa-m3/mole)
Group Method:	1.02E-004 atm-m3/mole (1.03E+001 Pa-m3/mole)
Henrys LC [via VP/WSol estimate using User-Entered or Estimated values]:	HLC: 6.767E-004 atm-m3/mole (6.856E+001 Pa-m3/mole) VP: 0.0497 mm Hg (source: MPBPVP) WS: 19 mg/L (source: WSKOWWIN)

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

Log Kow used:	3.96 (exp database)

Log Kaw used:	-1.376 (HenryWin est)
Log Koa (KOAWIN v1.10 estimate):	5.336
Log Koa (experimental database):	None

Probability of Rapid Biodegradation (BIOWIN v4.10):

Biowin1 (Linear Model): Biowin2 (Non-Linear Model) : Biowin3 (Ultimate Survey Model): Biowin4 (Primary Survey Model) : Biowin5 (MITI Linear Model) : Biowin6 (MITI Non-Linear Model): Biowin7 (Anaerobic Linear Model):	0.6443 0.9294 2.6935 (weeks-months) 3.6401 (days-weeks) 0.6190 0.5964 -0.0387
Ready Biodegradability Prediction:	NO

Hydrocarbon Biodegradation (BioHCwin v1.01):

Structure incompatible with current estimation method!

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

Vapor pressure (liquid/subcooled):	6.33 Pa (0.0475 mm Hg)
Log Koa (Koawin est):	5.336
Kp (particle/gas partition coef. (m3/ug)): Mackay model: Octanol/air (Koa) model:	4.74E-007 5.32E-008

Fraction sorbed to airborne particulates (phi):

Junge-Pankow model:	1.71E-005
Mackay model:	3.79E-005
Octanol/air (Koa) model:	4.26E-006

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

OVERALL OH Rate Constant =	94.7335 E-12 cm3/molecule-sec
Half-Life =	0.113 Days (12-hr day; 1.5E6 OH/cm3)
Half-Life =	1.355 Hrs

Ozone Reaction:

OVERALL Ozone Rate Constant =	43.000000 E-17 cm3/molecule-sec
Half-Life =	0.027 Days (at 7E11 mol/cm3)
Half-Life =	38.378 Min
Reaction With Nitrate Radicals May Be Important!	
Fraction sorbed to airborne particulates (phi): 2.75E-005 (Junge-Pankow, Mackay avg) 4.26E-006 (Koa method) Note: the sorbed fraction may be resistant to atmospheric oxidation	

Soil Adsorption Coefficient (KOCWIN v2.00):

Koc :	443.9 L/kg (MCI method)
Log Koc:	2.647 (MCI method)
Koc :	1122 L/kg (Kow method)
Log Koc:	3.050 (Kow method)

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

Total Kb for pH > 8 at 25 deg C:	1.022E-002 L/mol-sec
Kb Half-Life at pH 8:	2.148 years
Kb Half-Life at pH 7:	21.483 years

(Total Kb applies only to esters, carbmates, alkyl halides) **Volatilization from Water:** Henry LC: 0.000102 atm-m3/mole (estimated by Group SAR Method)

Half-Life from Model River:	9.472 hours
Half-Life from Model Lake:	220.8 hours (9.2 days)

Removal In Wastewater Treatment:

Total removal:	31.28 percent
Total biodegradation:	0.30 percent
Total sludge adsorption:	27.42 percent
Total to Air:	3.56 percent

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

	Mass Amount (percent)	Half-Life (hr)	Emissions (kg/hr)

Air	0.0392	0.517	1000
Water	15.8	900	1000
Soil	83.8	1.8e+003	1000
Sediment	0.389	8.1e+003	0

Persistence Time: 884 hr

The Ecological Categorization Results from the Canadian Domestic Substances List simply state that lovage oils (CAS RN 8016-31-7) are of uncertain persistence in the environment.

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

10.2. Aquatic toxicity

The Ecological Categorization Results from the Canadian Domestic Substances List simply state that lovage oils (CAS RN 8016-31-7) are not inherently toxic to aquatic organisms and are of low ecotoxicological concern.

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

ECOSAR version 1.11 reports the following aquatic toxicity data for CAS RN 8016-31-7:

Values used to Generate ECOSAR Profile:

Log Kow: 4.337 (EPISuite Kowwin v1.68 Estimate)

Wat Sol: 18.97 (mg/L, EPISuite WSKowwin v1.43 Estimate)

ECOSAR v1.11 Class-specific Estimations

Ester

ECOSAR Class	**Organism**	**Duration**	**End Pt**	**Predicted mg/L (ppm)**
Esters :	Fish	96-hr	LC50	1.133
Esters :	Daphnid	48-hr	LC50	1.833
Esters :	Green Algae	96-hr	EC50	0.539
Esters :	Fish		ChV	0.052
Esters :	Daphnid		ChV	0.640
Esters :	Green Algae		ChV	0.295
Esters :	Fish (SW)	96-hr	LC50	1.493
Esters :	Mysid	96-hr	LC50	0.464
Esters :	Fish (SW)		ChV	0.326

Esters :	Mysid (SW)		ChV	0.292
Neutral Organic SAR :	Fish	96-hr	LC50	1.284
(Baseline Toxicity) :	Daphnid	48-hr	LC50	0.904
	Green Algae	96-hr	EC50	1.644
	Fish		ChV	0.162
	Daphnid		ChV	0.161
	Green Algae		ChV	0.696

10.3. Sediment toxicity

No data available to us at this time.

10.4. Terrestrial toxicity

ECOSAR version 1.11 reports the following terrestrial toxicity data for CAS RN 8016-31-7:

Values used to Generate ECOSAR Profile:

Log Kow: 4.337 (EPISuite Kowwin v1.68 Estimate)

Wat Sol: 18.97 (mg/L, EPISuite WSKowwin v1.43 Estimate)

ECOSAR v1.11 Class-specific Estimations

Ester

ECOSAR Class	Organism	Duration	End Pt	Predicted mg/L (ppm)
Esters :	Earthworm	14-day	LC50	451.429*

Note: * = asterisk designates: Chemical may not be soluble enough to measure this predicted effect. If the effect level exceeds the water solubility by 10X, typically no effects at saturation (NES) are reported.

10.5. All other relevant types of ecotoxicity

EPISuite provides the following information on CAS RN 8016-31-7:

Bioaccumulation Estimates (BCFBAF v3.01):

Log BCF from regression-based method:	2.280 (BCF = 190.5 L/kg wet-wt)
Log Biotransformation Half-life (HL):	-0.5017 days (HL = 0.315 days)
Log BCF Arnot-Gobas method (upper trophic):	2.070 (BCF = 117.6)

Log BAF Arnot-Gobas method (upper trophic):	2.070 (BAF = 117.6)
log Kow used:	3.96 (expkow database)

The Ecological Categorization Results from the Canadian Domestic Substances List simply state that lovage oils (CAS RN 8016-31-7) are of uncertain bioaccumulative potential in the environment.

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

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

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

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

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

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

MATERIALS AND METHODS

Cigarette Design

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

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

TABLE 1
Blend composition of prototype cigarettes

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

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

Cigarette Performance

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

In Vitro Study Design

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

TABLE 2
Ingredients added to test cigarettes in study 1

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

(Continued on next page)

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

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

(Continued on next page)

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

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

(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.

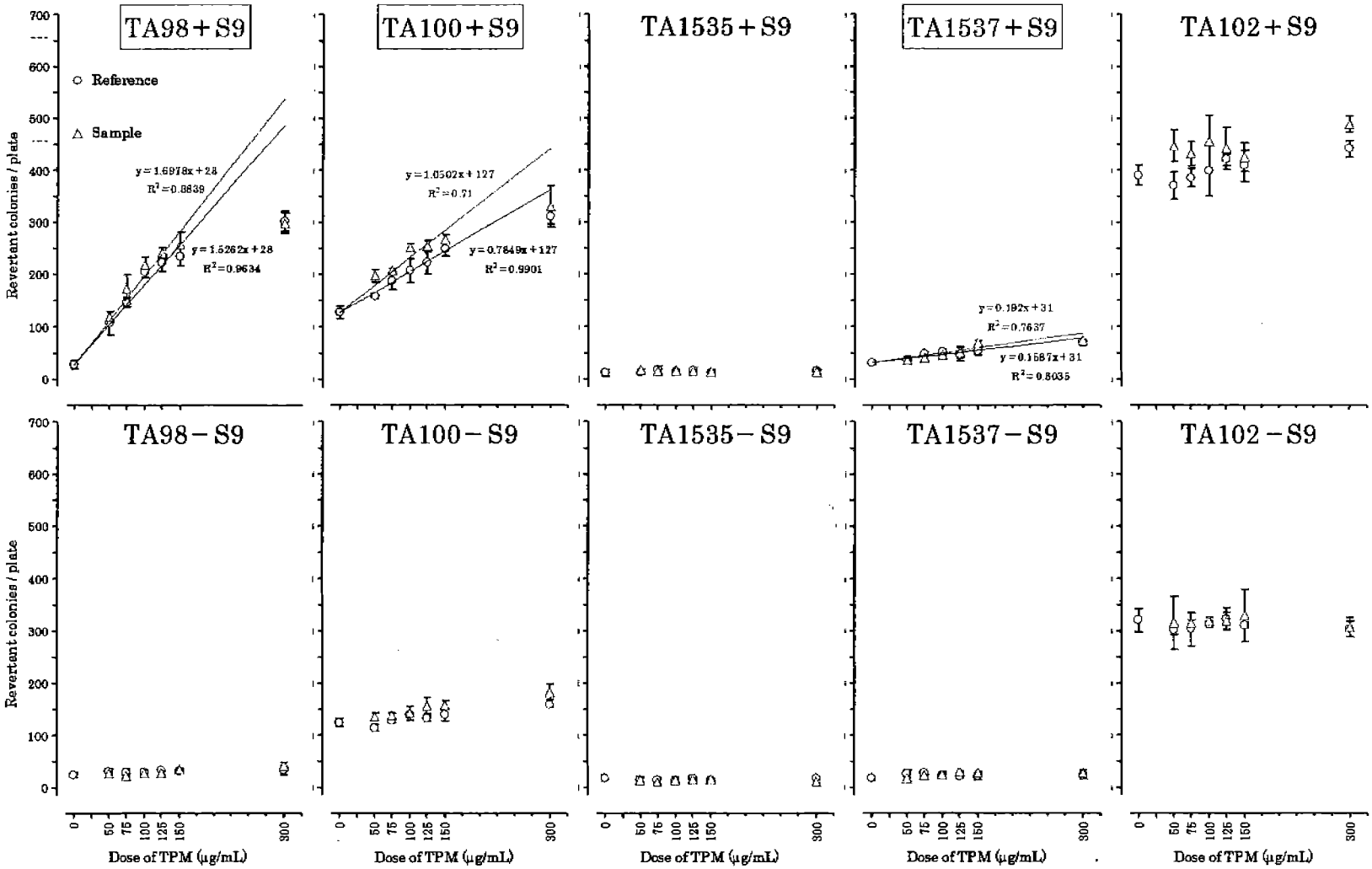
Body Weights and Clinical Observations

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.

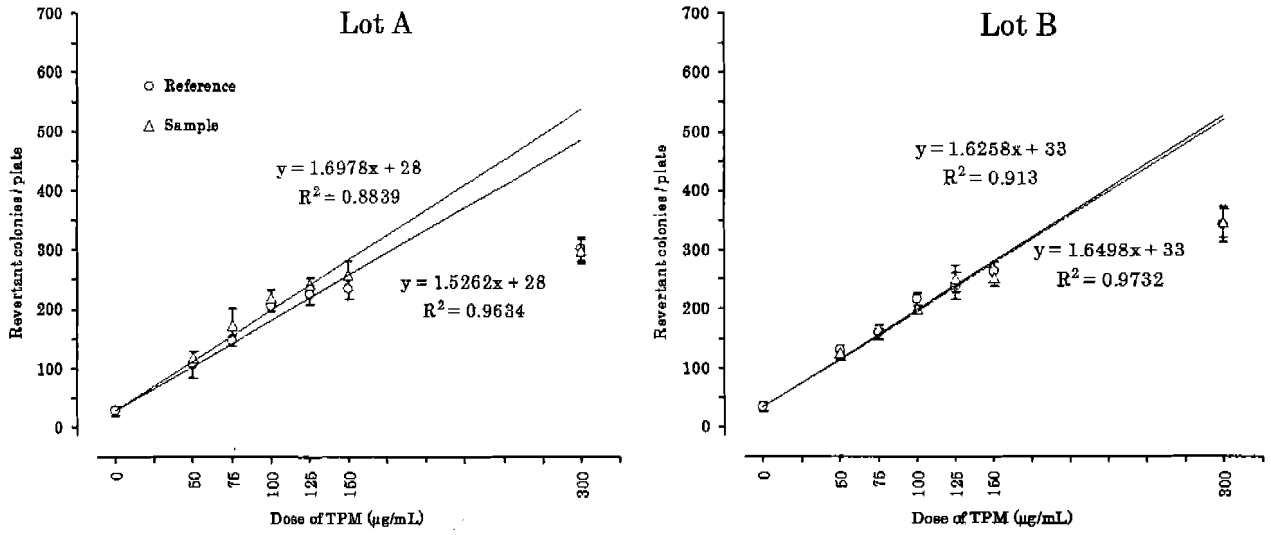
Organ Weights

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

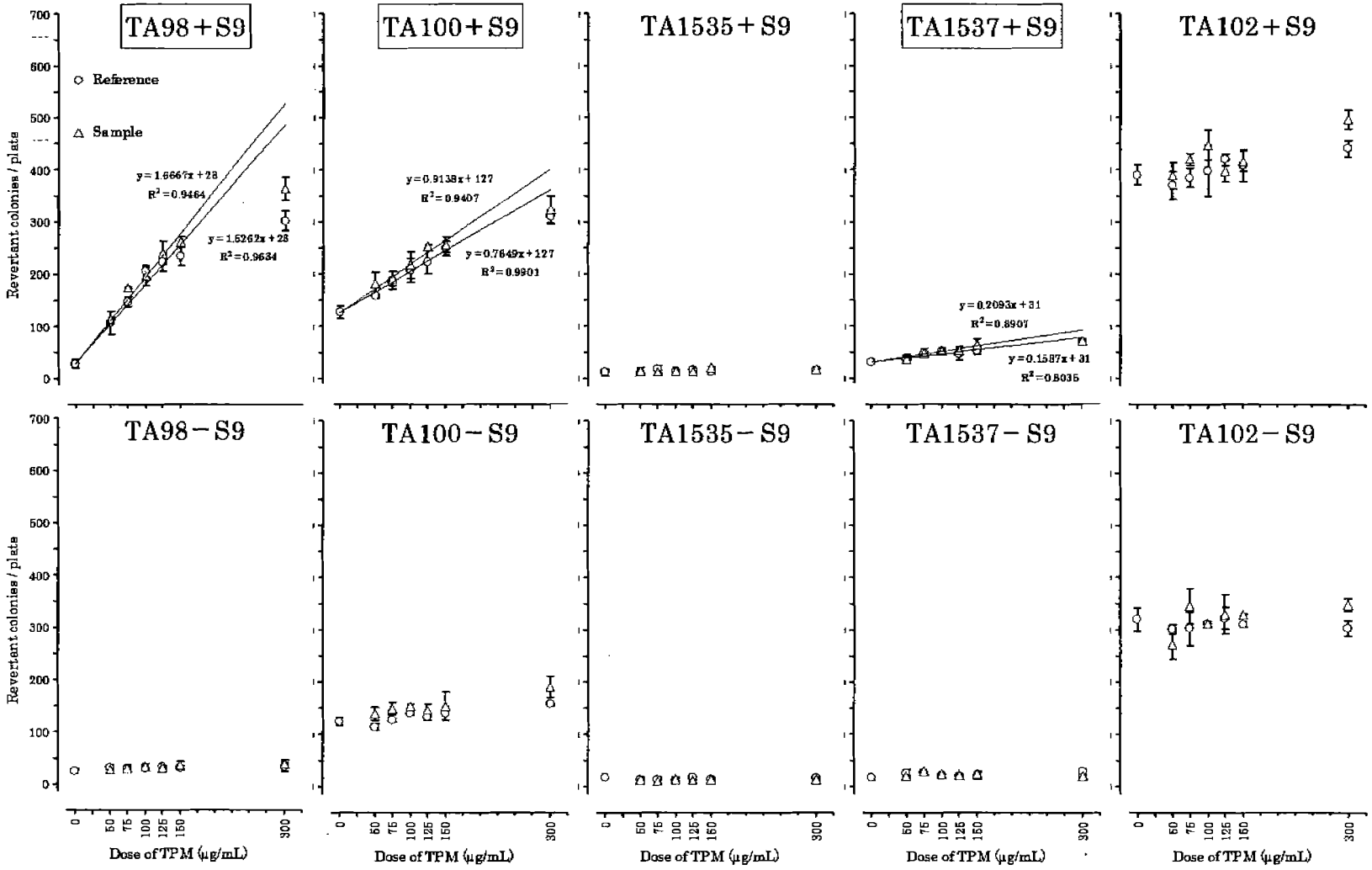
There were occasional statistically significant differences in hematology and clinical chemistry parameters from control values in groups exposed to smoke from test or reference cigarettes in both study 1 and study 2. These differences did not occur in a dose-response pattern and were well within ±2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



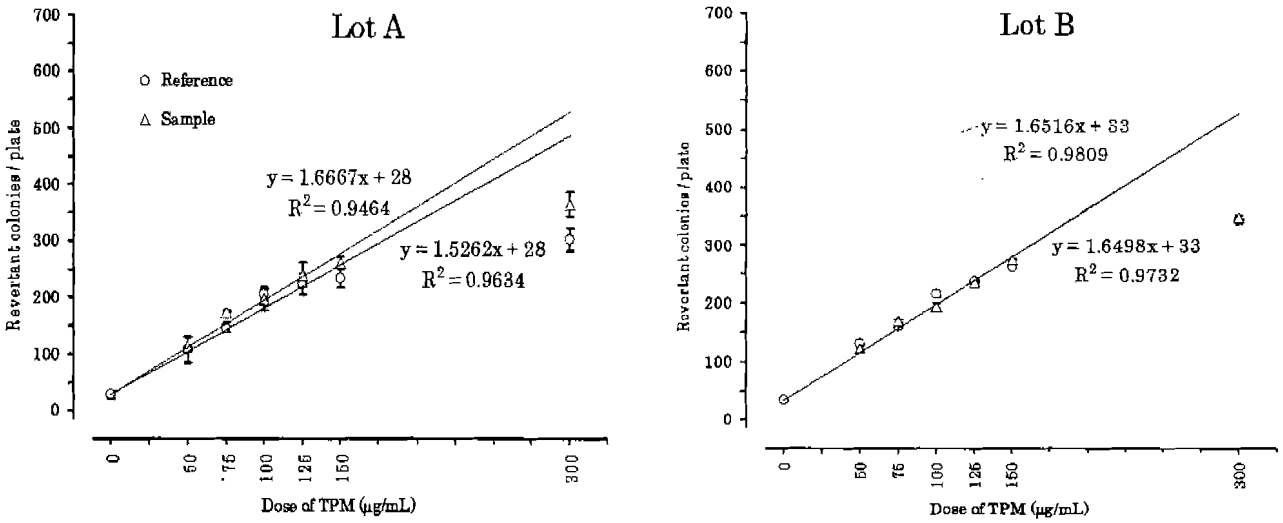
N=2. Only the first lot (Lot A) is indicated in this figure.
The second lot (Lot B) showed the same tendency as the first lot .

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

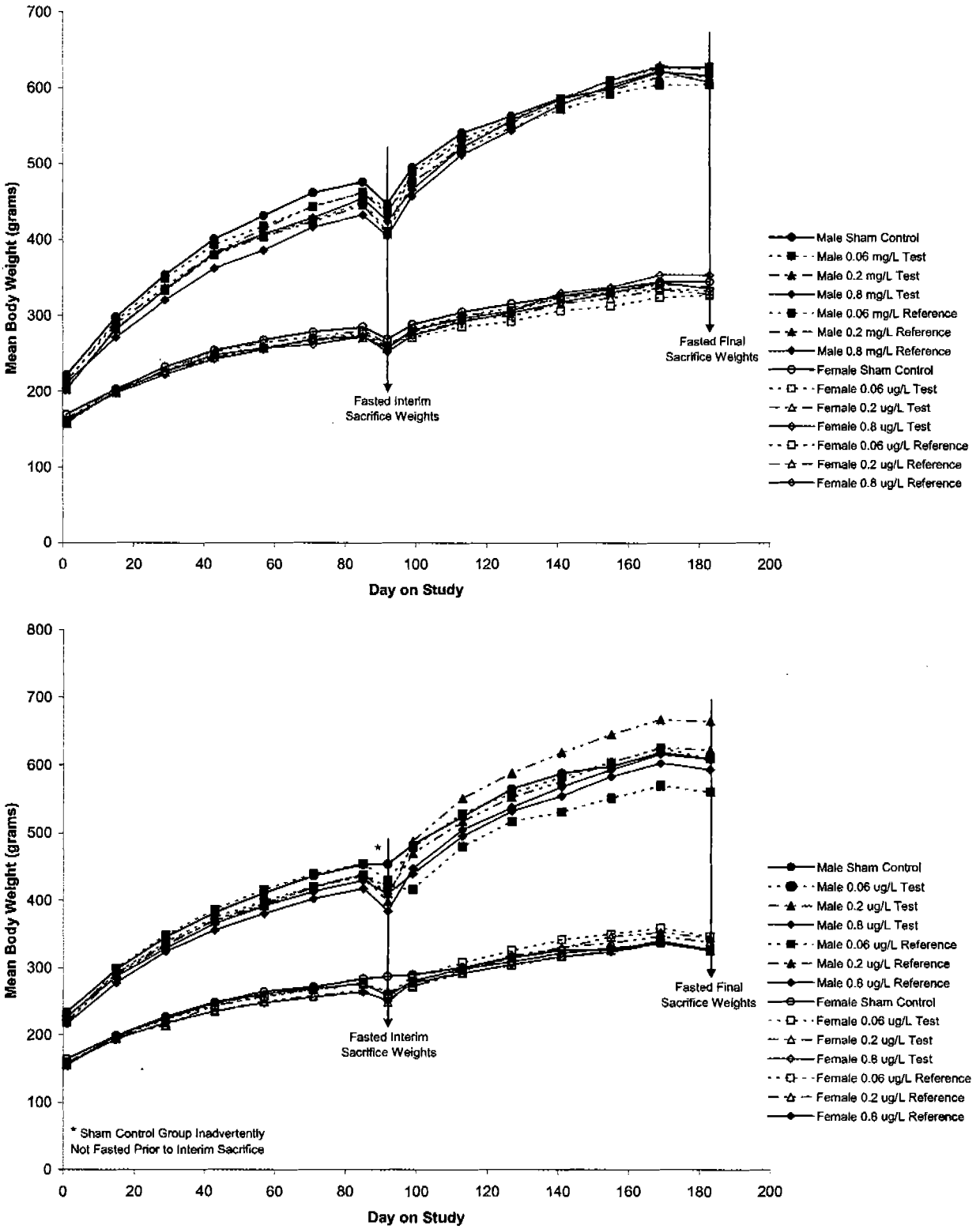


FIG. 5. Body weights, study 1 (top) and study 2 (bottom).

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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SCIENTIFIC OPINION

Scientific Opinion on the substantiation of health claims related to *Levisticum officinale* W.D.J. Koch and improvement of diuretic function (ID 2292, 3420) pursuant to Article 13(1) of Regulation (EC) No 1924/2006[1]

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA)[2]

European Food Safety Authority (EFSA), Parma, Italy

SUMMARY

Following a request from the European Commission, the Panel on Dietetic Products, Nutrition and Allergies was asked to provide a scientific opinion on a list of health claims pursuant to Article 13 of Regulation 1924/2006. This opinion addresses the scientific substantiation of health claims in relation to *Levisticum officinale* W.D.J. Koch and improvement of diuretic function. The scientific substantiation is based on the information provided by the Member States in the consolidated list of Article 13 health claims and references that EFSA has received from Member States or directly from stakeholders.

The food constituent that is the subject of the health claim is *Levisticum officinale* W.D.J. Koch. The Panel considers that *Levisticum officinale* W.D.J. Koch has been sufficiently characterised with the following conditions of use: dried root: 4-8 g/day, and equivalent quantity in extract.

The claimed effect "renal elimination/organism draining" is not sufficiently defined but from the proposed wordings the Panel assumes that the claimed effect relates to improvement of diuretic function. The Panel considers that no evidence has been provided to establish that improvement of diuretic function is beneficial to human health of the general population.

The Panel notes that the reference cited did not provide any scientific data that could be used to substantiate the claimed effect. The Panel concludes that a cause and effect relationship has not been established between the consumption of *Levisticum officinale* W.D.J. Koch and improvement of diuretic function.

KEY WORDS

Levisticum officinale W.D.J. Koch, diuretic function, health claim.

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1 On request from the European Commission, Question No EFSA-Q-2008-3025, EFSA-Q-2008-4149, adopted on 02 July 2009.

2 Panel members: Jean-Louis Bresson, Albert Flynn, Marina Heinonen, Karin Hulshof, Hannu Korhonen, Pagona Lagiou, Martinus Løvik, Rosangela Marchelli, Ambroise Martin, Bevan Moseley, Hildegard Przyrembel, Seppo Salminen, Sean (J.J.) Strain, Stephan Strobel, Inge Tetens, Henk van den Berg, Hendrik van Loveren and Hans Verhagen. Correspondence: nda@efsa.europa.eu

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The members of the Claims/Sub-Working Group Characterisation of Botanicals: Robert Anton, Luc Delmulle, Kirsten Pilegaard, Mauro Serafini and Hans Verhagen.

INFORMATION AS PROVIDED IN THE CONSOLIDATED LIST

The consolidated list of health claims pursuant to Article 13 of Regulation 1924/2006[3] submitted by Member States contains main entry claims with corresponding conditions of use and literature from similar health claims. The information provided in the consolidated list for the health claims subject to this opinion is given in Table 1.

Table 1. Main entry health claims related to *Levisticum officinale* W.D.J. Koch, including conditions of use from similar claims, as proposed in the Consolidated List.

ID	Food or Food constituent	Health Relationship	Proposed wording
2292	*Levisticum officinale* - common name: Lovage	Renal elimination / Organism draining	- Used to enhance the renal elimination of water; - used to facilitate the elimination functions of the organism; - used for the drainage of the organism; - stimulates the elimination function of the organism; - contributes to the functionary of the urinary tract.
	Conditions of use - Traditional use of the root / 4-8 g of roots per day / Equivalent quantity in extract.		
	Food or Food constituent	**Health Relationship**	**Proposed wording**
3420	*Levisticum officinale* - common name: Lovage	Renal elimination / Organism draining	- Traditionally used to enhance the renal elimination of water; - traditionally used to facilitate the elimination functions of the organism; - used to enhance the renal elimination of water; - used to facilitate the elimination functions of the organism; - used for the drainage of the organism; - diuretic effect; - stimulates the elimination function of the organism; - contributes to the functionary of the urinary tract.
	Conditions of use - Traditional use of the root / 4-8 g of roots per day / Equivalent quantity in extract.		

[3] Regulation (EC) No 1924/2006 of the European Parliament and of the Council of 20 December 2006 on nutrition and health claims made on foods. OJ L 404, 30.12.2006, p. 9–25.

ASSESSMENT

1. Characterisation of the food/constituent

The food constituent that is the subject of the health claim is *Levisticum officinale* W.D.J. Koch. The characterisation of *Levisticum officinale* W.D.J. Koch is performed by comparing data provided as conditions of use to information extracted from standard reference textbooks (see Table 2 below and Appendix C for list of standard reference textbooks used for the characterisation).

Table 2. Information on *Levisticum officinale* W.D.J. Koch from standard reference textbooks and the information provided as conditions of use.

ID	Scientific name	Part used	Nature of the preparation	Conditions of use
Text-book	***Levisticum officinale* W.D.J. Koch =** ***Angelica levisticum* All. = *Hipposelinum levisticum* W.D.J. Koch** **Apiaceae (Umbelliferae)**	**Herb; seed; root**	**Powder; essential oil; infusion**	**Herb, root:** **Powder: 4–8 g/day and equivalent preparations.** **Infusion: 2-4g/150mL water, several times/ day.** **Seed:** **Essential oil: 1-2 drops/day.**
2292	*Levisticum officinale* - common name: Lovage	Root	Root; extract *Note: It is assumed to be the dried root*	Root: 4-8g/day, and equivalent quantity in extract.
3420	*Levisticum officinale* - common name: Lovage	Root	Root; extract *Note: It is assumed to be the dried root*	Root: 4-8g/day, and equivalent quantity in extract.

The nature of preparation is not specified, but it is assumed to be the dried root.

The Panel considers that the food constituent, *Levisticum officinale* W.D.J. Koch, which is the subject of the health claim, has been sufficiently characterised with the following conditions of use: dried root: 4-8g/day, and equivalent quantity in extract.

2. Relevance of the claimed effect to human health

The claimed effect is "renal elimination/organism draining". The Panel assumes that the target population is the general population.

The claimed effect "renal elimination/organism draining" is not sufficiently defined. From the proposed wordings the Panel assumes that the claimed effect relates to improvement of diuretic function.

The Panel considers that no evidence has been provided to establish that improvement of diuretic function is beneficial to human health of the general population.

3. Scientific substantiation of the claimed effect

One reference was provided to substantiate the claimed effect. This was a monograph in which the claimed effect was stated. The Panel notes that the reference cited did not provide any scientific data that could be used to substantiate the claimed effect.

The Panel concludes that a cause and effect relationship has not been established between the consumption of *Levisticum officinale* W.D.J. Koch and improvement of diuretic function.

CONCLUSIONS

On the basis of the data presented, the Panel concludes that:

- The food constituent, *Levisticum officinale* W.D.J. Koch, which is the subject of the health claim, has been sufficiently characterised with the following conditions of use: dried root: 4-8 g/day, and equivalent quantity in extract.
- The claimed effect is "renal elimination/organism draining". The target population is assumed to be the general population. The Panel considers that no evidence has been provided to establish that improvement of diuretic function is beneficial to human health of the general population.
- A cause and effect relationship has not been established between the consumption of *Levisticum officinale* W.D.J. Koch and improvement of diuretic function.

DOCUMENTATION PROVIDED TO EFSA

Health claims pursuant to Article 13 of Regulation (EC) No 1924/2006 (No: EFSA-Q-2008-3025, EFSA-Q-2008-4149). The scientific substantiation is based on the information provided by the Member States in the consolidated list of Article 13 health claims and references that EFSA has received from Member States or directly from stakeholders.

The full list of supporting references as provided to EFSA is available on: http://www.efsa.europa.eu/panels/nda/claims/article13.htm

APPENDICES

APPENDIX A

BACKGROUND AND TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

The Regulation 1924/2006 on nutrition and health claims made on foods[4] (hereinafter "the Regulation") entered into force on 19th January 2007.

Article 13 of the Regulation foresees that the Commission shall adopt a Community list of permitted health claims other than those referring to the reduction of disease risk and to children's development and health. This Community list shall be adopted through the Regulatory Committee procedure and following consultation of the European Food Safety Authority (EFSA).

Health claims are defined as "any claim that states, suggests or implies that a relationship exists between a food category, a food or one of its constituents and health".

In accordance with Article 13 (1) health claims other than those referring to the reduction of disease risk and to children's development and health are health claims describing or referring to:

a) the role of a nutrient or other substance in growth, development and the functions of the body; or

b) psychological and behavioural functions; or

c) without prejudice to Directive 96/8/EC, slimming or weight-control or a reduction in the sense of hunger or an increase in the sense of satiety or to the reduction of the available energy from the diet.

To be included in the Community list of permitted health claims, the claims shall be:

(i) based on generally accepted scientific evidence; and

(ii) well understood by the average consumer.

Member States provided the Commission with lists of claims as referred to in Article 13(1) by 31 January 2008 accompanied by the conditions applying to them and by references to the relevant scientific justification. These lists have been consolidated into the list which forms the basis for the EFSA consultation in accordance with Article 13 (3).

ISSUES THAT NEED TO BE CONSIDERED

IMPORTANCE AND PERTINENCE OF THE FOOD[5]

Foods are commonly involved in many different functions[6] of the body, and for one single food many health claims may therefore be scientifically true. Therefore, the relative importance of food e.g. nutrients in relation to other nutrients for the expressed beneficial effect should be considered: for functions affected by a large number of dietary factors it should be considered whether a reference to a single food is scientifically pertinent.

It should also be considered if the information on the characteristics of the food contains aspects pertinent to the beneficial effect.

SUBSTANTIATION OF CLAIMS BY GENERALLY ACCEPTABLE SCIENTIFIC EVIDENCE

Scientific substantiation is the main aspect to be taken into account to authorise health claims. Claims should be scientifically substantiated by taking into account the totality of the available scientific data, and by weighing the evidence, and shall demonstrate the extent to which:

[4] OJ L12, 18/01/2007
[5] The term 'food' when used in this Terms of Reference refers to a food constituent, the food or the food category.
[6] The term 'function' when used in this Terms of Reference refers to health claims in Article 13(1)(a), (b) and (c).

(a) the claimed effect of the food is beneficial for human health,

(b) a cause and effect relationship is established between consumption of the food and the claimed effect in humans (such as: the strength, consistency, specificity, dose-response, and biological plausibility of the relationship),

(c) the quantity of the food and pattern of consumption required to obtain the claimed effect could reasonably be achieved as part of a balanced diet,

(d) the specific study group(s) in which the evidence was obtained is representative of the target population for which the claim is intended.

EFSA has mentioned in its scientific and technical guidance for the preparation and presentation of the application for authorisation of health claims consistent criteria for the potential sources of scientific data. Such sources may not be available for all health claims. Nevertheless it will be relevant and important that EFSA comments on the availability and quality of such data in order to allow the regulator to judge and make a risk management decision about the acceptability of health claims included in the submitted list.

The scientific evidence about the role of a food on a nutritional or physiological function is not enough to justify the claim. The beneficial effect of the dietary intake has also to be demonstrated. Moreover, the beneficial effect should be significant i.e. satisfactorily demonstrate to beneficially affect identified functions in the body in a way which is relevant to health. Although an appreciation of the beneficial effect in relation to the nutritional status of the European population may be of interest, the presence or absence of the actual need for a nutrient or other substance with nutritional or physiological effect for that population should not, however, condition such considerations.

Different types of effects can be claimed. Claims referring to the maintenance of a function may be distinct from claims referring to the improvement of a function. EFSA may wish to comment whether such different claims comply with the criteria laid down in the Regulation.

WORDING OF HEALTH CLAIMS

Scientific substantiation of health claims is the main aspect on which EFSA's opinion is requested. However, the wording of health claims should also be commented by EFSA in its opinion.

There is potentially a plethora of expressions that may be used to convey the relationship between the food and the function. This may be due to commercial practices, consumer perception and linguistic or cultural differences across the EU. Nevertheless, the wording used to make health claims should be truthful, clear, reliable and useful to the consumer in choosing a healthy diet.

In addition to fulfilling the general principles and conditions of the Regulation laid down in Article 3 and 5, Article 13(1)(a) stipulates that health claims shall describe or refer to "the role of a nutrient or other substance in growth, development and the functions of the body". Therefore, the requirement to describe or refer to the 'role' of a nutrient or substance in growth, development and the functions of the body should be carefully considered.

The specificity of the wording is very important. Health claims such as "Substance X supports the function of the joints" may not sufficiently do so, whereas a claim such as "Substance X helps maintain the flexibility of the joints" would. In the first example of a claim it is unclear which of the various functions of the joints is described or referred to contrary to the latter example which specifies this by using the word "flexibility".

The clarity of the wording is very important. The guiding principle should be that the description or reference to the role of the nutrient or other substance shall be clear and unambiguous and therefore be specified to the extent possible i.e. descriptive words/ terms which can have multiple meanings should be avoided. To this end, wordings like "strengthens your natural defences" or "contain antioxidants" should be considered as well as "may" or "might" as opposed to words like "contributes", "aids" or "helps".

In addition, for functions affected by a large number of dietary factors it should be considered whether wordings such as "indispensable", "necessary", "essential" and "important" reflects the strength of the scientific evidence.

Similar alternative wordings as mentioned above are used for claims relating to different relationships between the various foods and health. It is not the intention of the regulator to adopt a detailed and rigid list of claims where all possible wordings for the different claims are approved. Therefore, it is not required that EFSA comments on each individual wording for each claim unless the wording is strictly pertinent to a specific claim. It would be appreciated though that EFSA may consider and comment generally on such elements relating to wording to ensure the compliance with the criteria laid down in the Regulation.

In doing so the explanation provided for in recital 16 of the Regulation on the notion of the average consumer should be recalled. In addition, such assessment should take into account the particular perspective and/or knowledge in the target group of the claim, if such is indicated or implied.

TERMS OF REFERENCE

HEALTH CLAIMS OTHER THAN THOSE REFERRING TO THE REDUCTION OF DISEASE RISK AND TO CHILDREN'S DEVELOPMENT AND HEALTH

EFSA should in particular consider, and provide advice on the following aspects:

- Whether adequate information is provided on the characteristics of the food pertinent to the beneficial effect.
- Whether the beneficial effect of the food on the function is substantiated by generally accepted scientific evidence by taking into account the totality of the available scientific data, and by weighing the evidence. In this context EFSA is invited to comment on the nature and quality of the totality of the evidence provided according to consistent criteria.
- The specific importance of the food for the claimed effect. For functions affected by a large number of dietary factors whether a reference to a single food is scientifically pertinent.

In addition, EFSA should consider the claimed effect on the function, and provide advice on the extent to which:

- the claimed effect of the food in the identified function is beneficial.
- a cause and effect relationship has been established between consumption of the food and the claimed effect in humans and whether the magnitude of the effect is related to the quantity consumed.
- where appropriate, the effect on the function is significant in relation to the quantity of the food proposed to be consumed and if this quantity could reasonably be consumed as part of a balanced diet.
- the specific study group(s) in which the evidence was obtained is representative of the target population for which the claim is intended.
- the wordings used to express the claimed effect reflect the scientific evidence and complies with the criteria laid down in the Regulation.

When considering these elements EFSA should also provide advice, when appropriate:

- on the appropriate application of Article 10 (2) (c) and (d) in the Regulation, which provides for additional labelling requirements addressed to persons who should avoid using the food; and/or warnings for products that are likely to present a health risk if consumed to excess.

APPENDIX B

EFSA DISCLAIMER

The present opinion does not constitute, and cannot be construed as, an authorisation to the marketing of the food/food constituent, a positive assessment of its safety, nor a decision on whether the food/food constituent is, or is not, classified as foodstuffs. It should be noted that such an assessment is not foreseen in the framework of Regulation (EC) No 1924/2006.

It should also be highlighted that the scope, the proposed wordings of the claims and the conditions of use as proposed in the Consolidated List may be subject to changes, pending the outcome of the authorisation procedure foreseen in Article 13(3) of Regulation (EC) No 1924/2006.

APPENDIX C

FULL LIST OF STANDARD REFERENCE TEXTBOOKS USED FOR CHARACTERISATION PURPOSES

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EMEA (European Medicines Agency), HMPC community monographs, Committee on Herbal Medicinal Products. http://www.emea.europa.eu/htms/human/hmpc/hmpcmonographs.htm

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EMA/HMPC/524623/2011
Committee on Herbal Medicinal Products (HMPC)

Assessment report on *Levisticum officinale* Koch, radix

Based on Article 16d(1), Article 16f and Article 16h of Directive 2001/83/EC as amended (traditional use)

Final

Herbal substance(s) (binomial scientific name of the plant, including plant part)	*Levisticum officinale* Koch, radix
Herbal preparation(s)	Comminuted herbal substance
Pharmaceutical forms	Comminuted herbal substance as herbal tea for oral use
Rapporteur	Ewa Widy Tyszkiewicz
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1. Introduction

1.1. Description of the herbal substance(s), herbal preparation(s) or combinations thereof

- Herbal substance(s)

According to Toulemonde and Noleau (cited by Bradley 2006), the chemical composition of different extracts of *Levisticum officinale* revealed more than 190 volatiles, in prevalence monoterpene carbons and phtalides (Bylaite *et al.* 1998; 2000; Raal *et al.* 2008; Stahl-Biskup and Wichtmann 1991). They found, that n-butylidene-4,5-dihydrophtalide is the major constituent at 67% concentration range (Eskin and Tamir 2006). Cichy *et al.* (1984) after Bradley (2006) found in lovage roots phtalide dimers: levistolide A, levistolide B.

The volatile oil is present in the roots in amounts of 0.6-1%. Up to 70% of the oil consists of alkylphtalides which are mainly responsible for the characteristic odour (Wichtl 1994, 2004).Blank *et al.* (1993) described the presence of the flavour compound 3-hydroxy-4,5-dimethyl-2(5H)-furanone (sotolone) as responsible for intense odour which was described as ”hydrolyzed vegetable protein-like” or “curry-like”. Sotolone presence was recognised also in urine of patients with maple syrup urine disease leading to neurological damage and mental retardation (Podebrad *et al.* 1999).

Supercritical CO_2 extraction using constant pressure revealed the presence of *cis*-ligustilide (52.0%), trans-ligustilide (3.95%), 3n-butylidene phtalide E (1.75%), 3n-butylidene phtalide Z (0.73%), β-phellandrene (0.28%), α-terpinyl acetate (0.08%) (Daukšas *et al.* 2002). Moreover palmitic acid (2.81%), phytol (2.62%), linoleic acid (3.52%), stigmasterol (11%), and β-sitosterol (1.28%) were found (Daukšas *et al.* 2002).

Fehr (1980) identified the following substances in lovage root essential oil: α-pinen (4.5-4.6%), camphene (1.0-1.1%), β-pinene (7.1-8.0%), myrcene (0.9%), α-phellandrene 0.4-0.5%), α-terpinene (0.1%), limonene (0.8-1,2%), β-phellandrene (8.7-10.7%), *cis*-ocimene (0.2-0.4%), γ-terpinene/trans-ocimene (0.2-0.3%), terpinolene (1.2-1.5%), pentylcyclohexadiene (7.4-12.7%), pentylbenzene (0.1-0.3%), 3n-butytlidenephtalide (31.5-32.0%) and 3n-butylidene-4,5-dihydrophtalide (23.5-24.9%).

Phtalides are compounds that give characteristic flavours to some species of *Apiaceae*, including *Levisticum officinale* root. To date 71 Phtalides have been isolated from 40 species of the *Apiaceae* family (Naves 1943; Beck and Chou 2007; Nunes *et al.* 2009). By combining analytical and preparative separation methods Gijbels *et al.* (1980, 1982) identified E- and Z-butylidenephtalide, E- and Z-ligustilide, senkyunolide and validene-4,5-dihydrophtalide; *iso*senyunolide and propylidenephtalide. As the lead component of *Levisticum officinale* roots was described (*Z*)–Ligustilide (Segebrecht and Schilcher 1989). From Levisticum officinale roots 20 phtalides have been isolated and their content in the essential oil was 64-80%. Z-ligustilide (*cis*-3-n-butylidene-4,5-dihydrophtalide) was described by Mitsuhashi *et al.* (1960; 1966). Kobayashi *et al.* (1984; 1987) and they investigated phtalides in *Levisticum officinale* and described senkyunolide B, senkyunolide C, senkyunolide E, senkyunolide F, senkyunolide G, senkyunolide H, senkyunolide I and senkyunolide J. Other phtalides were identified after isolation by Liu *et al.* (2005): specifically *(E)*-3-butylidenephtalide, *(E)*-ligustilide and the dimer levistolide A. The simultaneous determination of ligustilide and butylidenphtalide using GC-MS-SIM was described by Chen *et al.* (2010) with tested ranges of 20-1,000 µg/ml for ligustilide and 2-100 µg/ml for butylidenphtalide.

According to Ezz El-Din and Hendawy (2010) lovage root oil is characterised by a high content of Z-ligustilide followed by falcarinol. In control plants the Z-ligustilide value was 23.4%, while after

fertilisation (chemical NPK) a value of 33.6% was obtained. The highest amount of falcarinol (32.6% vs: from 26.1% in control) resulted from plants fertilised with compost. In contrary, Najda and Wolski (2003) found that the monocyclic terpenes and phtalides comprised the major components of the essential oil from lovage roots.

The essential oil content in lovage roots depends on the harvesting time. Andruszczak (2007) observed that harvesting the leaves during the vegetation season has a negative influence on the accumulation of essential oil in the roots. Keeping the above ground parts of *Levisticum* until autumn, significantly increased the amount of essential oil from 0.52 to 0.85%.

According to Hogg (2001), literature sources present following composition of lovage root oil as % of oil yield - α-terpinyl acetate: 0.1-0.2, β-phellandrene: 1.7-15.5, α-phellandrene: 0.2-0.5, myrcene: 0.3-0.9, (Z)-ligustilide: 37.0-67.5 and pentylcyclohexadiene: 7.4-29.3.

Coumarins. Thin-Layer chromatographic analysis of lovage root methanolic extract provided semi-quantitative information on the presence of bergapten (R_f~0.6), angelicin (R_f~0.5), umbelliferone (R_f~0.45) and 3-butylidenphtalide at R_f~0.85. When compared to Imperatoriae radix and Angelicae radix, Levistici radix had a lower coumarin content (Wagner and Bladt, 2001). Using LC-DAD analysis of the chloroform extracts of lovage root, Paszkiewicz *et al.* (2008) detected both coumarins and furanocoumarins (psoralen and bergapten). Bradley (2006) estimates a total amount of coumarins of 3.2%, umbelliferone, coumarin and others (angelicin and scopoletin) included. Apterin (8-(glucosyloxy)isopropyll-9-hydroxy-8,9-dihydroangelicin) has been isolated in small amounts from nine plants of the *Apiaceae* family, *Levisticum officinale* enclosed (Fischer and Svendsen 1976).

Phenylpropanoids

Some amounts of chlorogenic, caffeic and ferulic acids were found (Baerheim Svendsen 1951; Bradley 2006).

Polyacetylenes

The aliphatic C17 polyacetylene falcarindiol was found by Cichy *et al.* (1984) and Zschocke *et al.* (1998) at the range of 0.14-0.2% of the dry drug. The ratio between Z-ligustilide and falcarindiol in lovage root was found to be (falcarindiol: Z-ligustilide) 1:2, Zschocke *et al.* (1998). The other polyacetylene Z-falcarinol was detected by Santos *et al.* (2005) with amounts between 19% and 46%. Moreover the presence of farnesene, phellandrene, elemene, heptanal and octanal was found.

- Herbal preparation(s)

Comminuted herbal substance

- Combinations of herbal substance(s) and/or herbal preparation(s) including a description of vitamin(s) and/or mineral(s) as ingredients of traditional combination herbal medicinal products assessed, where applicable.

Not applicable

1.2. Information about products on the market in the Member States

Regulatory status overview

Member State	Regulatory Status				Comments
Austria	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Belgium	☐ MA	☐ TRAD	☐ Other TRAD	☒ Other Specify:	Herbal substance only available in combination products
Bulgaria	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Cyprus	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Czech Republic	☐ MA	☐ TRAD	☐ Other TRAD	☒ Other Specify:	Herbal substance only available in combination products
Denmark	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Estonia	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Finland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
France	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Germany	☒ MA	☐ TRAD	☐ Other TRAD	☒ Other Specify:	Herbal substance only available in combination products
Greece	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Not known
Hungary	☐ MA	☒ TRAD	☐ Other TRAD	☒ Other Specify:	
Iceland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Ireland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Italy	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Latvia	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Liechtenstein	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Lithuania	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Luxemburg	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Malta	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
The Netherlands	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Norway	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Poland	☐ MA	☒ TRAD	☐ Other TRAD	☐ Other Specify:	
Portugal	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Not known
Romania	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Slovak Republic	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Slovenia	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Spain	☐ MA	☐ TRAD	☐ Other TRAD	☒ Other Specify:	Herbal substance only available in combination products

Sweden	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
United Kingdom	☐ MA	☐ TRAD	☐ Other TRAD	☒ Other Specify:	Herbal substance only available in combination products

MA: Marketing Authorisation
TRAD: Traditional Use Registration
Other TRAD: Other national Traditional systems of registration
Other: If known, it should be specified or otherwise add 'Not Known'
This regulatory overview is not legally binding and does not necessarily reflect the legal status of the products in the MSs concerned.

1.3. Search and assessment methodology

Databases assessed up to April 2011:

Science Direct, PubMed, Embase, Medline, Academic Search Complete, Toxnet

Search terms: *Levisticum officinale*, lovage root

2. Historical data on medicinal use

2.1. Information on period of medicinal use in the Community

Lovage has a long history: thousands of years of traditional medicinal use to treat a wide range of complaints (Colombo *et al.* 2011; De Voss 2010). The traditional use of *Levisticum officinale* in different diseases has been thoroughly documented in several handbooks and in folk tradition**.** Its use is mentioned in the ancient times by Dioscorides as Greek: *ligusticon*, Latin: *ligusticum*, the plant grown in the Alpine region of Liguria in Italy (Dioskurides).

Lovage preparations were used during the Middle Ages mainly as an emmenagogue, carminativum, diureticum and remedy for various skin ailments and were mentioned by Lonicerus (1564) and Matthiolus (1501-1577) according to Madaus (1938). The medieval sourcebook: the *Capitulary de Villis* (9th century) contains lovage as one of many culinary and medicinal plants that should be cultivated in every imperial garden (Arnold 1923). For centuries it is known as carminative and spasmolytic folk medicine. In the cosmetic/medical treatise of Trotula de Ruggiero of the Schola Medica Salernitana from the 11th century, garden lovage is indicated for skin lightening (Cavallo *et al.* 2008).

In Germany, it was approved in inflammatory conditions of the urinary tract and in kidney stones (Schimpfky 1900; Hogg 2001). In France, lovage was used as digestive and carminative (Goetz 2007) and as a confectionary ingredient.

The therapeutic activity of lovage is described in Madaus (1938) and in many other sources: Awang 2009; Berger 1960; Bradley 2006; Chevallier 1996; Duke 2002; Evans 2009; European Pharmacopoeia 5th and 6th ed.; Frohne 2006; Hänsel *et al.* 1994; Kohlmünzer 2000; Ożarowski 1976; Ożarowski and Jaroniewski 1987; Peter 2004; Raghavan 2007; Robbers and Tyler 1997; Roeske 1955; Schulz *et al.* 1998; Wagner and Wiesenauer 1995; Wiesenauer 2008; Weiss 1988; Weiss and Fintelman 2000; Wichtl 1994 and 2004; Wren 1975., Zehui and Watson 2005.

2.2. Information on traditional/current indications and specified substances/preparations

According to the information provided by the National Competent Authorities:

Czech Republic

Authorised combination products

Average number of combination substances: 3-5

The main combination substances are: Absinthii herba, Millefolii herba, Menthae piperitae herba, Levistici radix, Hyperici herba, Liquiritiae radix, Foeniculi fructus

Herbal tea for oral use;
indications: Traditionally used in temporary loss of appetite and mild gastrointestinal complaints such as bloating, and flatulence
on the market since 1995

Levistici radix has been a subject of Czechoslovak Pharmacopoeia since 1987,
recommended dosage in the last version of the Czech Pharmacopoeia (2009, Supplement 2010):
single dose 2 g, daily dose 4-8 g

Germany

Well-Established Use

One German standard marketing authorisation, herbal tea

The main combination substances are: Rosmarini folium, Ononidis radix

Combination products: In Germany there are 3 authorised combination products

Number of combination substances	Number of authorised combination products
2-3	2
4-5	1
>5	0

All preparations for which marketing authorisations for traditional use have been granted (with reference to former national regulations) are mentioned, regardless of the fact that some of them are not in accordance with current community law (as defined in Directive 2004/24/EC). Traditional preparations were authorised in 10-50% of well-established use doses when in parallel the same preparations were authorised under well-established use.

Poland

Traditional Use

Preparation: Comminuted herbal substance

On the market at least since 1967

Pharmaceutical form: Herbal teas (three)

Posology: Dose for decoction: 4-5 g in 1 cup (200 ml) of hot water/15 minutes.

Indications: To increase amount of urine to achieve flushing of the urinary tract.

Risks (adverse drug effects, literature)

Avoid an excessive exposure to the sun or UV light. Not recommended in hypersensitivity.

Spain

Combination products

A combination product has been submitted as a THMP (coated tablets) to the Spanish Agency, but at the moment it is still under assessment and therefore it isn't on the market yet.

United Kingdom

The herbal substance is only available in combination product.

A combination product was granted recently as a Traditional Herbal MP.

One coated tablet contains: *Levisticum officinale* Koch, radix, *Centaurium erythraea* Rafn. herba, *Rosmarinus officinalis* L., folium.

Indication: A traditional herbal medicinal product used to help flushing of the urinary tract and to assist in minor urinary complaints associated with cystitis in women only, based on traditional use only.

Search and assessment methodology

Databases assessed up to April 2011:
Science Direct, PubMed, Embase, Medline, Academic Search Complete, Toxnet

Search terms: *Levisticum officinale*, lovage root

2.3. Specified strength / posology / route of administration / duration of use for relevant preparations and indications

British Herbal Compendium (Bradley 2006)

Indications based on tradition: Inflammatory complaints of the lower urinary tract and renal gravel or lithuria. Menstrual disorders including dysmenorrhea, delayed menses, have menstrual bleeding and period pain. Digestive disorders including flatulent colic, heartburn and loss of appetite.

Contraindications: Pregnancy. Inflammatory disorders of the kidney; oedema due to impaired cardiac or renal function.

Side effects: none known

Interactions: None known

Dosage: Dried root, 1-3 g as an infusion or decoction; liquid extract 1:1 in 45% ethanol, 1-3 ml, up to 3 times daily.

British Herbal Pharmacopoeia (1983)

Indications: flatulent colic, dyspepsia, oedema, renal, dysmenorrhea, delayed menses, lithuria, cystitis

Dosage: Dose of 0.5-2 g as decoction in water or milk. Liquid extract 1:1 in 45% alcohol.
Dose 0.5-2 ml.

Duration of use: no information

Commission E Monograph. Levistici officinale L.
(Bundesanzeiger No 101, published June 1, 1990)

Indications: Irrigation therapy for inflammation of the lower urinary tract and for prevention of kidney gravel.

Contraindications: Preparations of lovage should not be used if acute inflammation of the kidney parenchyma with impaired kidney function exists.

No irrigation therapy in cases of oedema due to limited heart and kidney function.

Side effects: None known

Interactions with other drugs: None known

Dosage: Unless otherwise prescribed: 4-8 g of drug and equivalent preparations daily.

Duration of use: no information

Hagers Handbuch der Pharmazeutischen Praxis (Hänsel et al. 1994)

Indications: Irrigation therapy for inflammation of the lower urinary tract and for prevention of kidney gravel.

Side effects: Allergic contact reactions.

Warnings: With prolonged use of lovage root, exposure to ultraviolet light and intense sun bathing should be avoided.

Contraindications: Not to be used in cases of acute inflammation of the kidney or with impaired kidney function. No irrigation therapy in cases of oedema due to impaired heart and kidney functions.

Dosage: Tea: 1.5 g in a 150 ml of hot water, steep for 10-15 minutes, then strain. Drink between meals.

Daily dose: 4-8 g of the drug

Duration of use: no information.

Herbal Drugs and Phytopharmaceuticals (Wichtl 2004)

Indications: For irrigation therapy in cases of inflammatory diseases of the lower urinary tract and for prevention of kidney gravel

Contraindications: Should not be used in cases of acute inflammation of the kidney parenchyma or with impaired kidney function. No irrigation therapy in cases of oedema due to limited heart and kidney functions.

Side effects: None known

Interactions: None known

Dosage: The daily dosage is 4-8 g of dried root or corresponding preparations.

Note: For use in irrigation therapy, abundant fluid intake is necessary.

Warning: With prolonged use of lovage root, exposure to ultraviolet light and intense sun bathing should be avoided.

Duration of use: no information

Herbal Medicine (Weiss and Fintelman 2000).

Indications: As a diuretic for treatment of unspecific inflammatory diseases of the efferent urinary passages and renal gravel.

Daily dose: Pour one cup of boiling water onto one to two teaspoonful of the finely chopped drug, cover and allow to steep for 10-15 minutes, then strain. Drink one cup of the hot tea before meals, several times a day.

Duration of use: No information

Lehrbuch der Biologischen Heilmittel (Madaus 1938)

Indications: Diuresis to treat oedema, inflammation of the lower urinary tract and prevention of kidney gravel. As carminative to improve digestion, as expectorant and emmenagogue.

Dosage: Orally: 5-8 g in hot water (cup). Drink up to 3-4 times daily.

Maximum dose is not described.

Duration of use: No information

Medicinal Plants of the World (Wyk and Wink 2004)

Indications: Inflammation of the lower urinary tract. Kidney gravel, oedema.

Traditionally: Used as stomachic and carminative, as expectorant and emmenagogue.

Mode of use: A tea is made by pouring boiling water over 1.5-3 g of the dry herb.

Drink two or three times a day as a diuretic, half an hour before a meal as stomachic.

Duration of use: No information.

Medicinal Herbs: A Compendium (Gehrmann et al. 2005)

Indications: Cleansing therapy with bacterial and inflammatory illness of urinary tract, as a prophylaxis for kidney gravel; also for dyspeptic complaints such as indigestion, heartburn, feelings of fullness, flatulence

Dosage: 2-4 g (1 teaspoon)/150 ml , 10-15 minutes, 1 cup several times/day between meals; stomachic: 1 cup 30 minutes before meals

Daily dose: 4-8 g

Warning: Ensure sufficient fluid intake, minimum 2 litre/day

Contraindications: Inflammatory diseases of the kidneys or urinary drainage passages, reduced cardiac and renal function; pregnancy

Side effects: Individual cases of photodermatosis; long-therapy → avoid exposure to direct sunlight or intensive UV radiation

Duration of use: No information

Normdosen gebräuchlicher Arzneistoffe und Drogen(Haffner et al. 2009)

Oral route: Drug, 2 g 2-3 times daily; Extractum siccum: 0.3 g, 2-3 times daily, Extractum fluidum: 2 g, 2-3 times daily

PDR for Herbal Medicines, Gruenwald et al. editors (2000, 2004)

The medicinal parts are the dried rhizome and roots.

Indications: Infections of the urinary tract. Kidney and bladder stones.

Irrigating therapy for inflammation of the urinary tract and irrigating therapy for prevention of kidney gravel.

Mode of use: Comminuted herb for internal use.

Tea is prepared by using 2-4 g drug to 1 cup, several times a day between meals.

Contraindications: Not to be used during pregnancy

Side effects: The drug possesses a low potential for sensitisation. An elevation of UV-sensitivity among light-skinned people is possible (Phototoxic effect of the furocoumarins).

Duration of use: No information.

Receptariusz Zielarski (1967)

Indications: Stomachicum, diureticum, carminativum

Dosage: Decoctum: 1 tablespoon of the drug in 1 glass of hot water

Oral route: Drink half a glass (100 ml) two-three times daily

Ziołolecznictwo, Ożarowski (1976)

Indications: Irrigating therapy for inflammation of the urinary tract.

Dosage: Decoctions: 10-20 g of the drug in 400 ml of hot water.

Drink half a glass (100 ml) three times daily

Duration of use: No information

3. Non-Clinical Data

3.1. Overview of available pharmacological data regarding the herbal substance(s), herbal preparation(s) and relevant constituents thereof

In vitro experiments

Lovage root essential oil

Antimicrobial activity

According to Deans and Ritchie (1987) after Ceylan and Fung (2004) lovage essential oil showes antibacterial activity against both Gram positive and Gram-negative bacteria and is one of ten essential oils (thyme, cinnamon, bay, clove, almond–bitter, pimento, marjoram, angelica, nutmeg) with strongest activity from total 25 essential oils tested.

The fractionated methanolic extract of *Levisticum officinale* (10 g) was tested against bacterial strains of isolates of Gram-negative bacteria (Garvey *et al.* 2011). The extract was fractionated to active compounds yielding falcarindiol (450 mg), levistolide A (69 mg) and oleic and linoleic acids. The lovage extract showed synergistic activity with five antibiotics (ciprofloxacin, tetracycline, chloramphenicol,

erythromycin and ethidium bromide) against several *Salmonella typhimurium* isolates with innate efflux pump multidrug resistance AcrAB-TolC and was found the most active from eighty four extracts from 21 plants. Bioassay screening was carried out with ciprofloxacin in the absence or presence of the chloroform extract of *Levisticum officinale* on agar plates. A zone of inhibition of the ciprofloxacin (0.5, 1 and 2 mg/l) plus plant extract (100 mg/l) larger than that of antibiotic alone was estimated as synergy (Table 1). However no synergism was observed with the fractions and purified substances, implying that a composition of active substances is needed for efflux inhibition.

Table 1. Minimum inhibitory concentrations (MICs) of ciprofloxacin (CIP) in the absence and presence of Levisticum officinale extracts against Gram-negative bacteria (modified after Garvey et al. 2011)

Strain	MIC (mg/l)[b]	
	CIP	CIP+Lo
S. typhimurium L354	0.03	**0.008**
S. typhimurium L828	0.03	**0.008**
S. typhimurium L 829	0.008	**0.004**
S. typhimurium L3	0.008	**0.002**
S. typhimurium L10	0.06	**0.03**
Enterobacter cloacae A1	0.12	**0.06**
Serratia marcescens B14	0.06	0.06
Pseudomonas aeruginosa G1	1	1
Klebsiella pneumoniaeH42	0.06	**0.03**
Escherichia coli I114	0.06	**0.03**
Morganella morgani J29	0.015	**0.008**

S. typhimurium, *Salmonella enterica* serotype *typhimurium*; Lo, chloroform extract of *Levisticum officinale* extract.
[b]Bold text indicates synergistic combinations (i.e. MICs for the combination lower than for the antibiotic alone).

Antimycobacterial activity

The dichloromethane extract of the root of *Levisticum officinale* was tested in a microtiter plate dilution method against *Mycobacterium fortuitum* and *Mycobacterium aurum* with a MIC of 64 µg/ml. Further fractionation resulted in two active polyacetylenes: (**1**) 3(*R*)-falcarinol and (**2**) 3(*R*)-8 (S)-falcarindiol with MICs against *M. fortuitum*, 30.4 µm (**1**), 16.4 µm (**2**) and against *M. aurum*, 60.8 µm (**1**) , 16.4 µm (**2**). MICs of standard chemotherapeutics were as follows: ethambutol: 115.5 µm (*M. fortuitum*) and 14.6 µm (*M. aurum*); isoniazid: 3.4 µm (*M. fortuitum*) and 29.2 µm (*M. aurum*), respectively (Schinkovitz *et al.* 2008).

Inhibition of activity of pancreatic lipase

The methanolic extract of lovage roots (20 g of powdered herbal substance with 200 ml of absolute methanol) inhibited by 55% the activity of pancreatic lipase at a concentration range of 0.05-0.15 mg/ml of the extract (Gholamhoseinian *et al.* 2010).

Isolated constituents of lovage essential oil

Antifungal activity

Polyacetylenes of falcarindiol type constitutively present in *Apiaceae* have been identified as antifungal substances acting as prevention against infections (Garrod *et al.* 1979). They inhibit spore germination of different fungi at the concentration range of 20-200 µg/ml. It has also been shown that falcarindiol have anti-inflammatory, antiplatelet, and cytotoxic activity (Christensen and Brandt 2006b).

Acetylenes from *Apiaceae* have been shown to be toxic to bacteria and fungi and play a role in resistance and protection of plants against infection. Kemp (1978) found a total inhibition of spore germination using falcarindiol against *Alternaria brassicicola* and *Septoria nodorum* at a concentration of 20 µg/ml. However falcarinol even at the concentration of 200 µg/ml did not affect the fungi themselves.

The polyacetylene falcarindiol has been identified as a phytoalexin in tomato fruits and leaves infected by fungi (Christensen and Brandt 2006a).

Hadacek and Greger (2000) tested the activity of falcarindiol in various dilutions and diffusion bioassays against three selected plant filamentous microfungi, *Botrytis cinerea*, *Cladosporium herbarum* and *Fusarium avenaceum*. MIC defined as the lowest concentration/spot causing mycelium-free zones was found as follows: for *Botrytis cinerea*: 25 µg/ml, for *Cladosporium herbarum:* 12 µg/ml and for *Fusarium avenaceum:* 50 µg/ml.

Spasmolytic activity

Ligustilide in concentration dependent manner relaxed isolated rat mesenteric artery rings preconstricted with potassium chloride. *In vitro* experiments showed that the β-receptors, ATP sensitive potassium channels, calcium activated potassium channels and inwardly rectifying potassium channels were not involved in the myorelaxation. It was found, that ligustilide (10, 30, 100 µM) concentration-dependently (more than 10 µM) inhibited vasoconstrictive effects of Na and $CaCl_2$ in Ca^{2+} -free medium. The pD_2 value of ligustilide (the negative logarithm of the drug concentration that elicited 50% relaxation) to $CaCl_2$ was 4.45±0.02. Contractions induced by caffeine were also inhibited, therefore the ryanodine receptors were involved through inhibition of intracellular Ca^{2+} release. The authors conclude that the vasorelaxant effect of ligustilide in rat mesenteric artery is related to inhibition the voltage-dependent calcium channel and receptor-mediated calcium ryanodine receptors (Cao *et al.* 2006).

In *in vitro* experiments Ko (1980) tested the spasmolytic activity of **butylidenephtalide** in comparison to the papaverine activity in isolated guinea pig ileum, guinea pig vas deferens and guinea pig taenia coli. Butylidenephtalide non competitively inhibited contractions induced by ACh, K^+ and Ba^{2+} in normal Tyrode solutions and to administered exogenous Ca^{2+} in high K^+, Ca^{2+} free Tyrode solution. However butylidenephtalide pD_2 values were significantly inferior to those of papaverine ($p<0.001$). In the author's opinion butylidenephtalide probably inhibits the Ca^{2+} release from the cellular membrane and from the intracellular calcium storage and/or inhibits the Ca^{2+} influx from the extracellular fluid.

In other experiments Ko *et al.* (1997) separated two geometric isomers, the *Z*- and the *E*- forms of synthetic butylidenephtalide and checked their inhibition of voltage-dependent calcium channels in depolarised guinea-pig ileum longitudinal smooth muscle. It was found that ***E*- butylidenephtalide** (2-100 µM) inhibited contractions with a pD_2 value of 4.56±0.18. **Z- butylidenephtalide** non-competitively induced significantly lower inhibition of Ca^{2+} as compared to E-butylidene phtalide with contractions at the range 50-100 µM and the pD_2 value of 3.88±0.20 ($p<0.05$).

Butylidenephtalide inhibited the calcium release from calcium stores in isolated rat aortic rings probably due to an independent mechanism not related to the production of inositol-1,4,5-trisphosphate (Ko *et al.* 1998).

Butylidenephtalide induced a concentration-dependent (1-300 µM) vasorelaxing effect in the rat isolated aorta constricted by use of **(1)** 60 mM of kalium chloride (KCl) and **(2)** 30 nM of 9,11-dideoxy-9α,11α-methanoepoxyprostaglandin H_2 (EC_{50} is the effective concentration of the test compound to cause 50% of its maximal response; **(1)** - EC_{50}: 4.00±0.03, n=5; **(2)** -EC_{50}: P 4.29±0.03, n=5, respectively (Chan *et al.* 2006). The authors suggest that the spasmolytic effect is dependent on the modulation of the L-type voltage operated and prostanoid receptor operated Ca^{2+} channels.

Both **ligustilide** and **senkyunolide A** induced vasorelaxation effects in rat isolated aorta with cumulative concentrations within a range of 1 – 300 µM (Chan *et al.* 2007). Both compounds had a similar spasmolytic activity against contractions induced by 9,11-dideoxy-9α,11α-methanoepoxyprostaglandin $F_{2α}$, phenylephrine, 5-hydroxytryptamine and KCl with pD_2: 4.14±0.08, pD_2: 4.39±0.11, pD_2 4.56±0.12, pD_2: 4.43±0.08, n=6, respectively (Chan *et al.* 2007).

Ligustilide inhibited spontaneous periodic contractions of the isolated rat uterus in a concentration dependent manner (EC_{50}=4.4±2.7-6.1) µg/ml and antagonised prostaglandin $F_{2α}$ (95.3% at 8 µg/ml) and acetylcholine induced contractions (73.9% at 8 µg/ml) (Du *et al.,* 2006).

From another experiment performed in the rat isolated aorta model, Chan *et al.* (2009) reported a synergistic myorelaxant activity with NO-donor sodium nitroprusside in the rat isolated aorta constricted by use of 9, 11-dideoxy-9α,11α-methanoepoxyprostaglandin H_2. According to the authors this relaxant synergism is related to the modulation of the Ca^{2+} sensitisation-mediated tone.

Antiproliferative activity

Liu *et al.* (2011) tested the inhibitory effects of **n-butylidenphtalide** on proliferation *in vitro* and *in vivo* in the model of balloon injured a rat carotid artery on neointimal hyperplasia. In the cell culture of a rat aorta derived cell line, **n-butylidenphtalide** at concentrations of 25-100 µg/ml significantly inhibited the proliferation and arrested the cell cycle in the G_o/G_1 phase. Treatment with **n-butylidenphtalide** (150-300 mg/kg) significantly reduced the proliferation of the intima compared to the control group in rats with balloon injured carotid artery 2 weeks after injury. Immunohistochemical tests revealed a significant decrease of the proliferative activity in the 60-300 mg/kg treated rats. In contrary, the apoptotic activity was significantly increased in animals receiving 60-300 mg/kg of **n-butylidenphtalide**. The authors suggest that dose dependent up-regulation of the Nur77 gene (nerve growth factor IB) implicated in cell growth/survival and apoptosis is related to the antiproliferative activity of **n-butylidenphtalide**.

Inhibition of 5-lipoxygenase (5-LO) products synthesis

Falcarindol blocks 5-LO product synthesis at the range of IC_{50} concentrations 2-10 µM (Alanko *et al.* 1994; Schneider and Bucar 2005; Werz 2007).

Inhibition of TNF-α

Liu *et al.* (2005) described the dose and time dependent inhibition of the transcription of TNF-α mRNA by **Z-ligustilide** and **senkyunolide A** in monocytes. Moreover, the two phtalides suppressed the TNF-α mediated NF-κB activation.

GABAergic activity

Deng *et al.* (2006) have shown that a new phtalide dimer **gelispirolide** composed of Z-ligustilide and Z-butylidenephtalide induces inhibitory effects on the binding of [^{3}H] diazepam to the GABAa receptors with IC_{50} values of 29 µM.

In vivo experiments

Lovage root and lovage root essential oil

Diuretic activity

Lovage root is used as diuretic in urinary tract infections (Bag *et al.* 2008; Combest *et al.* 2005; Yarnell 2002).

Butylidenphtalide and ligustilide possess spasmolytic properties (Wichtl 1994, 2004).

Early experiments performed in rabbits and mice (Vollmer and Weidlich 1937; Vollmer and Hübner 1937) with an infusion of *Levistici radix* showed a slight increase of the urine volume and the concentration of chloride ions. However, in previous tests lovage root (0.25-1 g of crude drug per animal) , did not induce diuresis (Bradley 2006).

According to Vollman (1988), diuretic effects of the oil is due to activity of the terpene derivatives.

Oestrogenic activity

San Martin (1958) observed in experiments performed *in vivo* with female ovariectomised rats estrogenic effects on the vagina and on the uterus (the production of cornified epithelial cells in the vaginal smear of a castrated animal) after subcutaneous injection of the aqueous extract (1: 8) of *Levisticum officinale*. According to the Allen-Doisy criterion (measures of vaginal cornification as endpoint) estrogenic effects were seen after lovage extract administration with an activity of 1 g drug equivalent to 8 IU of estradiol. In comparison, according to San Martin (1958) 1 g of *Humulus lupulus* extract induces estrogenic effects equivalent to 200-300 UI of estradiol.

Isolated constituents of lovage essential oil

Analgesic activity

In two mice models, the acetic acid-induced writhing response and the formalin-induced licking time, ligustilide given intragastrically significantly and dose-dependently reduced the writhing response and licking time (Du *et al.* 2007). **Ligustilide** at the dose 10 mg/kg induced the same range of analgesia as a very high dose of aspirin (200 mg/kg) in the acetic acid induced writhing movements.

Neuroprotective activity.

Using the model of forebrain ischemia/reperfusion injury in mice, Kuang *et al.* (2006) demonstrated for **ligustilide** significant protection against brain damage. Transient ischemia was produced by the bilateral common carotid artery occlusion. After intraperitoneal administration, ligustilide dose dependently significantly decreased the infarction volume of the brain tissue. The infarction volume was without ligustilide: 22.1±2.6%, after 5 mg/kg: 11.8±5.2%($p<0.05$) and after 20 mg/kg: 2.60±1.5% ($p<0.01$).

Antiproliferative activity

Liu *et al.* (2011) tested the inhibitory effects of **n-butylidenphtalide** on proliferation *in vitro* and *in vivo* in the model of balloon injured a rat carotid artery on neointimal hyperplasia. In the cell culture of a rat aorta derived cell line, **n-butylidenphtalide** at concentrations of 25-100 µg/ml significantly inhibited the proliferation and arrested the cell cycle in the G_o/G_1 phase. Treatment with **n-butylidenphtalide** (150-300 mg/kg) significantly reduced the proliferation of the intima compared to the control group in rats with balloon injured carotid artery 2 weeks after injury. Immunohistochemical tests revealed a significant decrease of the proliferative activity in the 60-300 mg/kg treated rats. In contrary, the apoptotic activity was significantly increased in animals receiving 60-300 mg/kg of **n-butylidenphtalide**. The authors suggest that dose dependent up-regulation of the Nur77 gene (nerve growth factor IB) implicated in cell growth/survival and apoptosis is related to the antiproliferative activity of **n-butylidenphtalide**.

3.2. Overview of available pharmacokinetic data regarding the herbal substance(s), herbal preparation(s) and relevant constituents thereof

No data are available concerning lovage root on pharmacokinetics due to its complex phytochemical composition.

Ligustilide intranasally administered rapidly enters the central nervous system through the nasal cavity (Guo *et al.* 2009). In contrary, after oral administration only 2.6% is absorbed in the rat (Yan *et al.* 2008). Ligustilide can be detected in brain tissue samples already after 5 minutes of the application.

Overview of pharmacokinetics

Due to lack of data on pharmacokinetics of lovage root no conclusions can be drawn.

3.3. Overview of available toxicological data regarding the herbal substance(s)/herbal preparation(s) and constituents thereof

No published studies could be found concerning reproductive and development toxicity, carcinogenicity and immunotoxicity of lovage root.

Due to the weak estrogenic activity, lovage extract appears of concern for reproductive and developmental toxicity. In the absence of sufficient data the use during pregnancy and lactation is not recommended.

Acute toxicity and skin irritation

Tisserand and Balacs (1998) published a summary of data on the acute oral toxicity and the skin irritation of lovage root oil. In their opinion, the oil is non-toxic and is safe to use unless there are other specific reasons: rodent oral LD_{50} values are in the range 2-5 g/kg, and caus a very mild irritation of the skin at >5 g/kg.

Cytotoxicity

No data concerning cytotoxicity of the herbal substance are available.

The cytotoxic activity of polyacetylenes present in *Apiaceae* vegetables was tested *in vitro* against different human cancer cell lines: CEM-C7H2, T-ALL – acute lymphoblastic leukaemia, U937- human histiocytic lymphoma, HRT-18 and HT-2912, colorectal carcinoma cell lines (Zidorn *et al.* 2005). **Falcarinol** and **falcarindiol** exhibited medium level cytotoxicity against leukaemia, lymphoma and

myeloma tested cell lines in the range of IC_{50} approximately 30 µM. However falcarinol was more active against CEM-C7H2 line with IC_{50} value of 3.5 µM. Activity of falcarindiol against HRT-18 and HT-2912 was in the range of IC_{50} >100 µM, but IC_{50} of falcarinol against HRT-18 was 42.3 µM and against HT2912 – 63.9 µM.

Falcarinol in low concentrations (0.5-10 µM) increased significantly the proliferation of CaCo cell line and decreased expression of caspase-3 with decreased basal DNA strand breakage (Young *et al.* 2007). Contrary, in higher concentrations. twenty µM falcarinol induced an increase of caspase activity and decreased proliferation of the CaCo cell line.

Genotoxicity and mutagenicity

Bergapten and lovage extract exhibited strong **photomutagenicity** in an arginine-requiring (Arg^+) mutant strain of green algae *Chlamydomonas reinhardtii* (Schimmer 1983; Schimmer *et al.* 1980). Bergapten was tested for photomutagenicity under long-wave ultraviolet light (NUV). A bergapten concentration of 5 µg/ml, with application of NUV (dose of 2-2.7 W/m^2, fluence rate of 2.7 W/m^2 for 10 to 15 minutes) resulted with a maximum number of Arg^+ from 1 400 to 3 000 revertants/10^8 surviving cells (Schimmer *et al.* 1980). Some experiments with preincubation in the dark enhanced the number of revertants. In this conditions bergapten induced >1100 revertants per 10^8 surviving cells (UVA dose of 3 kJ/m^2, fluence rate 5.1 $W/m^{2)}$ (Schimmer 1997).

Levistici radicis extract (0.25%), (NUV dose 2W/m2 with fluence rate 7.2 kJ/m^2 for 60 minutes) induced the number of 7 revertants/10^8 surviving cells (Schimmer 1983).

Photoactivated furanocoumarins (psoralens) are linked to gene mutations and chromosomal aberrations. They have been shown as mutagenic and carcinogenic (Bruneton 1995, Diawara *et al.* 1999). In the absence of ultraviolet light the toxicity of furanocoumarins is low, with an LD_{50} in mammals of 300 to 600 mg/kg body weight. However, even 1 mg/kg body weight in humans can be harmful in the presence of UV radiation. The lowest observed adverse effect (LOAEL) was 0.14-0.38 mg/kg body weight. Therefore, furanocoumarins intake should be limited (Hsu and Friedlander 2010; Schulzova *et al.* 2007).

Regulatory status

Levisticum officinale extract has been recognised as GRAS (Botanicals Generally Recognized As Safe (http://www.biologie.uni-hamburg.de/b-online/ibc99/dr-duke/gras.htm) for use as a flavour ingredient. Presently lovage extract is used at levels below 100 ppm in selected brands of cigarettes. It is administered directly to the tobacco and can undergo pyrolysis when smoked.

EFSA Scientific Cooperation (ESCO, 2009) in 'Compendium of botanicals that have been reported to contain toxic, addictive, psychotropic, or other substances of concern' classifies the toxicity of substances present in lovage roots and recommends restrictions for use for: coumarin, furocoumarins (mainly bergapten, umbelliferone, psoralen); root seeds: imperatorin 12.82 mg/kg, 5-methoxypsoralen 6.38 mg/kg, psoralen 3.8 mg,kg, 8-methoxypsoralen 0.5 mg/kg.

3.4. Overall conclusions on non-clinical data

The published data on pharmacological activities support the traditional use of preparations containing lovage root in the proposed indication.

However, despite daily intake of lovage root as common vegetable, the therapeutic importance of the plant can be overestimated.

Levisticum officinale root oil is relatively nontoxic following acute exposure both by oral or topical administration.

Adequate genotoxicity studies have not been performed. Due to the presence of furanocoumarins, photoactivation by UV radiation is seen as a concern.

No published data could be found on the carcinogenicity of the lovage root and the lovage root preparations.

Lovage root use is not recommended during pregnancy and lactation. Moreover, some caution is needed in combination with UV radiation exposure due to possible photoactivation caused by furanocoumarins

4. Clinical Data

4.1. Clinical Pharmacology

4.1.1. Overview of pharmacodynamic data regarding the herbal substance(s)/preparation(s) including data on relevant constituents

There are no data on human pharmacodynamics.

4.1.2. Overview of pharmacokinetic data regarding the herbal substance(s)/preparation(s) including data on relevant constituents

There are no data on human pharmacokinetics.

4.2. Clinical Efficacy

4.2.1. Dose response studies

There are no specific data available on dose-response studies.

4.2.2. Clinical studies (case studies and clinical trials)

None were published on mono-preparations of lovage root.

4.2.3. Clinical studies in special populations (e.g. elderly and children)

No information available.

4.3. Overall conclusions on clinical pharmacology and efficacy

There are no data available from controlled clinical studies, therefore the medicinal use of *Levisticum officinale* root is not suitable for well-established use authorisation.

5. Clinical Safety/Pharmacovigilance

5.1. Overview of toxicological/safety data from clinical trials in humans

There are no adverse effects reported from the Member States, however allergic reactions to the *Apiaceae* family should be considered, particularly with UV exposure.

Concerns regarding phototoxicity are not supported by clinical data or pharmacovigilance signals to be relevant for the use of lovage root as recommended in the monograph.

There are no data from clinical trials available.

5.2. Patient exposure

None reported.

5.3. Adverse events and serious adverse events and deaths

Allergy. The extensive handling of lovage during harvest under prolonged exposure to strong sunlight induced dermatitis within a few hours with itching and erythema (Wolf 1995). After 36 hour on exposed arms and legs bullae and vesicles were formed with marked hyperpigmentation after 3 weeks. In the fresh lovage specimens appreciable amounts of furanocoumarins were found: 3.12 ±0.64 and 4.02±0.64 µg/g wet weight ± SE) for psoralen and 5-methoxypsoralen, respectively (Ashwood-Smith *et al.* 1992). A similar case of dermatitis was described Vollman (1988) after contact with lovage oil.

Laboratory findings

No data available.

5.4. Safety in special populations and situations

There are no reports of use *Levisticum officinale* root in children. The use of lovage root is not recommended in children and adolescents younger than 18 years of age.

Drug interactions

None reported for *Levisticum officinale* preparations.

Coumarin present in the plant, devoid of anticoagulant activity, can be transformed e.g. by moulding to the anticoagulant dicoumarol. Abnormal clotting values and bleeding can be expected after drinking the herbal tea prepared from several plants with coumarin as active constituent present (Aronson 2009).

A theoretical risk for potentiation activity of warfarin exists, as lovage root contains coumarin or coumarin derivatives, bergapten and imperatorin which inhibit platelet aggregation (Ebadi 2007; Heck *et al.* 2000; Herr 2005; Nutescu 2006; Patel and Gohil 2008; Shehadeh 2007).

Moreover, herbal products containing lovage should be discontinued in advance in patients undergoing surgery (Heyneman 2003).

Use in pregnancy and lactation.

Lovage root should not be used during pregnancy and lactation.

Levisticum officinale is in the list of plants that should not be used during pregnancy because of their potential uterine stimulating and emmenagogue properties (Belew 1999; Ernst 2002). However this recommendation is discussed and questioned (Guba 2000).

Chuchupate lovage (*Ligusticum porteri*, *Apiaceae*), but not *Levisticum officinale*, was used by Spanish and Mexicans in New Mexico as an emmenagogue and abortifacient (Conway and Slocumb 1979).

Overdose

None reported.

Effect on ability to drive or operate machinery or impairment of mental ability

None reported.

5.5. Overall conclusions on clinical safety

The allergic reactions in patients allergic to *Apiaceae* should be considered.

Concerns regarding phototoxicity are not supported by clinical data or pharmacovigilance signals to be relevant for the oral use of lovage root as recommended in the monograph.

6. Overall conclusions

The available data are sufficient to include the traditional use of specified preparations of lovage root in a monograph of the European Community. *Levisticum officinale* root fulfils the requirement of therapeutic use for at least 30 years (15 years within the Community, Directive 2004/24/EC).

Indication: Traditional herbal medicinal product to increase the amount of urine to achieve flushing of the urinary tract as an adjuvant in minor urinary complaints.

Due to the lack of data on mutagenicity and carcinogenicity toxicity, a list entry for *Levisticum officinale* root cannot be recommended.

Benefit / risk assessment

There are some concerns about side effects with *Levisticum officinale* root due to presence of furanocoumarins (psoralens) or interaction with oral anticoagulants.

There are reported side effects concerning allergic reactions due to the contact with *Levisticum officinale*, particularly after prolonged exposure to strong sunlight or UV radiation.

Concerns regarding phototoxicity are not supported by clinical data or pharmacovigilance signals to be relevant for the use of lovage root as recommended in the monograph.

No serious adverse events with a therapeutic posology of the herbal preparations are reported.

Despite the insufficiency of toxicological data base, levels of exposure associated with the use of lovage root most probably do not result in significant risk to human health.

It can be concluded that the benefit/risk assessment for *Levisticum officinale* preparations is positive for use as an adjuvant in minor urinary complaints.

Annex

List of references

Lovage (*Levisticum officinale*)

Synonyms / Common Names / Related Terms

4,5-Dimethyl-3-hydroxy-2[5H]-furanone (sotolone), β-phellandrene, α-Pinene, α-phellandrene/myrcene, ache de montagne, anjodan romi, aplo de Montana, badekraut, bladder seed, carvacrol eugenol céleri perpétuel, Cornish lavage, d-terpineol, devesil, garden lovage, gaya à tige simple, Goritsvet, gulyavitsa, harilik leeskputk, Italian lovage, lavas, legústico, lestyán, leuştean, leuşzean, levístico, *Levisticum officinale*, levistiko, liebstöckl, libeček, libeček lékařský, ligustico, liperi, lipstikka, livèche, ljekoviti ljupčac, lova, love parsley, løpstikke, løvstikke, lubbestok, lubczyk ogrodowy, luibh an liugair, lupstājs, lusch, luststock, maggikraut, maggiplant, magi-začin, mankracht, n-butyl-phthalide, n-butylidene phthalide old english lavage, rabaji, rabeji, reobwiji, robaji, robejji, robiji, robwiji, sea lovage, sedanonic anhydride, sedano di montagna, sedano di monte, selen, sirenas, siunas, skessujurt, vaistinė gelsvė, yuan xie gang gui, yuan ye dang gui, yuhn yihp dong gwai.

Mechanism of Action

Pharmacology:

- **Constituents**: The chemicals found in lovage oil are mainly phthalides and terpenoids, including n-butylidene phthalide n-butyl-phthalide, sedanonic anhydride, d-terpineol, carvacrol eugenol, and volatile acids (1).
- Lovage also contains many volatile chemicals including 4,5-dimethyl-3-hydroxy-2[5H]-furanone (sotolone), β-phellandrene, α-Pinene and α-phellandrene/myrcene.[3],[4]
- Lovage also contains coumarin.[2]

Pharmacodynamics/Kinetics:

- Insufficient available evidence.

References

1. Simon JE, Chadwick.A.F., Craker E. Herbs: An Indexed Bibliography. 1971-1980. *The Scientific Literature on Selected Herbs, and Aromatic and Medicinal Plants of the Temperate Zone* 1984.
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3. Podebrad F, Heil M, Reichert S, et al. 4,5-dimethyl-3-hydroxy-2[5H]-furanone (sotolone)--the odour of maple syrup urine disease. *J Inherit Metab Dis* 1999;22(2):107-114. 10234605
4. Bylaite E, Roozen JP, Legger A, et al. Dynamic deadspace-gas chromatography-olfactometry analysis of different anatomical parts of lovage (Levisticum officinale Koch.) at eight growing stages. *J Agric Food Chem* 2000;48(12):6183-6190. 11312790

1. IDENTIFICATION OF THE SUBSTANCE/MIXTURE AND THE COMPANY/UNDERTAKING

1.1. Identification of the substance/mixture

Trade name: **LOVAGE OIL**
Substance name: LOVAGE OIL
CAS Number: 84837-06-9
CE Number: 284-292-7

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

Raw material for the manufacture of fragrances and/or flavourings.

1.3. Details of the supplier of the safety data sheet

Company: **Ernesto Ventós SA**
Address: Carretera Real, 120 B
08960 Sant Just Desvern – Barcelona – SPAIN
Telephone: (00 34) 934 706 210
Fax: (00 34) 934 733 010
E-mail: info@ventos.com

1.4. Emergency telephone number

NCEC (+44) 1865 407333 (24h)
NCEC (+34) 91 114 2520 (24h) (ES)
NCEC (+1) 202 464 2554 (24h) (USA, Canada)

2. HAZARDS IDENTIFICATION

2.1. Classification of the substance or mixture

Flammable Liquids - Category 4 - H227
Acute Toxicity - Category 5 (oral) - H303
Skin Irritant - Category 3 - H316
Hazardous to the aquatic environment, short-term (acute) - Category 3 - H402
Hazardous to the aquatic environment, long-term (chronic) - Category 3 - H412

2.2. Label Elements

Signal Word:
Warning

Hazard statements:
H227 – Combustible liquid.
H303 – May be harmful if swallowed.
H316 – Causes mild skin irritation.
H412 – Harmful to aquatic life with long lasting effects.

Precautionary statements:
P210 – Keep away from heat/sparks/open flames/hot surfaces. — No smoking.
P312 – Call a POISON CENTER or doctor/physician if you feel unwell.
P332+P313 – If skin irritation occurs: Get medical advice/attention.

2.3. Other hazards

No Information available

3. COMPOSITION/INFORMATION ON INGREDIENTS

3.1. Substances

Chemical name: LOVAGE OIL
CAS number: 84837-06-9
EC number: 284-292-7

Hazardous constituents:

Chemical Name	% (w/w)	CAS No EC No	Classification according to GHS
PHTALIDES	≥50	- -	Acute Toxicity - Category 4 (oral) - H302
...	...	...	...

...	...	...	...
BETA-PINENE	≥0.1; <1	127-91-3 204-872-5	Flammable Liquids - Category 3 - H226 Skin Irritant - Category 2 - H315 Skin sensitizer - Category 1B - H317 Aspiration hazard - Category 1 - H304 Hazardous to the aquatic environment, short-term (acute) - Category 1 - H400 Hazardous to the aquatic environment, long-term (chronic) - Category 1 - H410
ALPHA-PINENE	≥0.1; <1	80-56-8 201-291-9	Flammable Liquids - Category 3 - H226 Acute Toxicity - Category 4 (oral) - H302 Skin Irritant - Category 2 - H315 Skin sensitizer - Category 1B - H317 Aspiration hazard - Category 1 - H304 Hazardous to the aquatic environment, short-term (acute) - Category 1 - H400 Hazardous to the aquatic environment, long-term (chronic) - Category 1 - H410
LIMONENE	≥0.1; <1	138-86-3 205-341-0	Flammable Liquids - Category 3 - H226 Skin Irritant - Category 2 - H315 Skin sensitizer - Category 1B - H317 Aspiration hazard - Category 1 - H304 Hazardous to the aquatic environment, short-term (acute) - Category 1 - H400 Hazardous to the aquatic environment, long-term (chronic) - Category 1 - H410

See the full text of the hazard statements in section 16.

3.2. Mixtures
Not applicable.

4. FIRST-AID MEASURES

4.1. Description of necessary first aid measures
Ingestion: Rinse mouth with water.
Obtain medical advice.
Keep at rest. Do not induce vomiting.

Eye contact: In case of contact with eyes, rinse immediately with plenty of water for at least 15 minutes and seek medical advice.

Inhalation: Remove person to fresh air and keep at rest.
Seek immediate medical advice.

Skin contact: Take off immediately all contaminated clothing.
Thoroughly wash affected skin with soap and water.
Seek medical attention if symptoms persist.

4.2. Most important symptoms and effects, both acute and delayed
No information available.

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

5. FIRE-FIGHTING MEASURES

5.1. Extinguishing Media
Water spray, carbon dioxide, dry chemical powder or appropriate foam.
For safety reasons do not use full water jet.

5.2. Special hazards arising from the substance or mixture
Known or Anticipated Hazardous Products of Combustion: Emits toxic fumes under fire conditions.

5.3. Advice for firefighters
High temperatures can lead to high pressures inside closed containers.
Avoid inhalation of vapors that are created. Use appropriate respiratory protection.
Do not allow spillage of fire to be poured into drains or watercourses.
Wear self-contained breathing apparatus and protective clothing.

6. ACCIDENTAL RELEASE MEASURES

6.1. Personal precautions, protective equipment and emergency procedures
Evacuate surronding areas. Ensure adequate ventilation. Keep unnecessary and unprotected personnel from entering.
Do not breathe vapor/spray. Avoid contact with skin and eyes. Information regarding personal protective measures: see section 8.

6.2. Environmental precautions
To avoid possible contamination of the environment, do not discharge into any drains, surface waters or groundwaters.

6.3. Methods and materials for containment and cleaning up

Cover with an inert, inorganic, non-combustible absorbent material (e.g. dry-lime, sand, soda ash).
Place in covered containers using non-sparking tools and transport outdoors.
Avoid open flames or sources of ignition (e.g. pilot lights on gas hot water heater).
Ventilate area and wash spill site after material pickup is complete.

6.4. Reference to other sections

Information regarding exposure controls, personal protection and disposal considerations can be found in sections 8 and 13.

7. HANDLING AND STORAGE

7.1. Precautions for safe handling

Do not store or handle this material near food or drinking water. Do not smoke.
Avoid contact with the eyes, skin and clothing. Wear protective clothing and use glasses.
Observe the rules of safety and hygiene at work.
Keep in the original container or an alternative made from a compatible material.

7.2. Conditions for safe storage, including any incompatibilities

Store in tightly closed and preferably full containers in a cool, dry and ventilated area, protected from light.
Keep away from sources of ignition (e.g. hot surfaces, sparks, flame and static discharges).
Keep away from incompatible materials (see section 10).

7.3. Specific end use(s)

No information available.

8. EXPOSURE CONTROLS AND PERSONAL PROTECTION

8.1. Control parameters

Components with occupational exposure limits:
None known.

8.2. Exposure controls

Measures should be taken to prevent materials from being splashed into the body.
Provide adequate ventilation, according to the conditions of use. Use a mechanical exhaust if required.

8.3. Individual protection measures, such as personal protective equipment

Eye/Face protection:	Chemical safety goggles are recommended. Wash contaminated goggles before reuse.
Hand Protection:	Chemical-resistant gloves are recommended. Wash contaminated gloves before reuse.
Body protection:	Personal protective equipment for the body should be selected based on the task being performed and the risks involved.
Respiratory Protection:	In case of insufficient ventilation, use suitable respiratory equipment.
Environmental exposure controls:	Emissions from ventilation or process equipment should be checked to ensure they comply with environmental protection legislation. In some cases, filters or engineering modifications to the process equipment will be necessary to reduce emissions to acceptable levels.

9. PHYSICAL AND CHEMICAL PROPERTIES

9.1. Information on basic physical and chemical properties

Appearance:	Liquid
Colour:	Conforms to standard
Odour:	Conforms to standard
Odour theshold:	Not determined
pH:	Not determined
Melting point/freezing point:	Not determined
Boling point/boiling range:	Not determined
Flash point:	62 °C
Evaporation rate:	Not determined
Flammability:	Not determined
Lower flammability/Explosive limit:	Not determined
Upper flammability/Explosive limit:	Not determined
Vapour pressure:	Not determined
Vapour Density:	Not determined
Density:	1,022—1,06 g/mL (20°C)
Relative density:	1,022—1,06 (20°C)
Water solubility:	INSOLUBLE IN WATER

Solubility in other solvents:	SOLUBLE IN ETHANOL
Partition coefficient n-octanol/water:	Not determined
Auto-ignition temperature:	Not determined
Decomposition temperature:	Not determined
Viscosity, dynamic:	Not determined
Viscosity, kinematic:	Not determined
Explosive properties:	Not determined
Oxidising properties:	Not determined

10. STABILITY AND REACTIVITY

10.1. Reactivity
No hazardous reactions if stored and handled as prescribed/indicated.

10.2. Chemical stability
The product is stable if stored and handled as prescribed/indicated.

10.3. Possibility of hazardous reactions
No hazardous reactions if stored and handled as prescribed/indicated.

10.4. Conditions to Avoid
Conditions to Avoid: Excessive heat, flame or other ignition sources.

10.5. Incompatible materials
Avoid contact with strong acids and bases and oxidizing agents.

10.6. Hazardous decomposition products
During combustion may form carbon monoxide and unidentified organic compounds.

11. TOXICOLOGICAL INFORMATION

Acute toxicity	May be harmful if swallowed.
Skin corrosion/irritation	Causes mild skin irritation.
Serious eye damage/irritation	Based on the data available, the criteria for classification are not met.
Respiratory or skin sensitisation	Based on the data available, the criteria for classification are not met.
Germ cell mutagenicity	Based on the data available, the criteria for classification are not met.
Carcinogenicity	Based on the data available, the criteria for classification are not met.
Reproductive toxicity	Based on the data available, the criteria for classification are not met.
STOT-single exposure	Based on the data available, the criteria for classification are not met.
STOT-repeated exposure	Based on the data available, the criteria for classification are not met.
Aspiration hazard	Based on the data available, the criteria for classification are not met.

12. ECOLOGICAL INFORMATION

12.1. Toxicity
Assessment:
Harmful to aquatic life with long lasting effects.
Experimental/calculated data:
No information available.

12.2. Degradability
No information available.

12.3. Bioaccumulative potential
No information available.

12.4. Soil mobility
No information available.

12.5. Other adverse effects
See also sections 6, 7, 13 and 15
Do not allow to get into waste water or waterways.

13. DISPOSAL CONSIDERATIONS

13.1. Waste treatment methods
Dispose of in accordance with national and local environmental regulations.

14. TRANSPORT INFORMATION

	ADR/RID/ADN	IMDG	IATA-ICAO
14.1. UN Number	Not classified as hazardous goods	Not classified as hazardous goods	Not classified as hazardous goods
14.2. UN Proper Shipping Name	Not applicable	Not applicable	Not applicable
14.3. Transport Hazard Class(es)	Not applicable	Not applicable	Not applicable
14.4. Packing Group	Not applicable	Not applicable	Not applicable
14.5. Environmental hazards	No	No	No
Additional information			

14.6 Special precautions for user
None known

14.7. Transport in bulk according to Annex II of MARPOL 73/78 and the IBC Code
No information available

15. REGULATORY INFORMATION

15.1. Safety, health and environmental regulations/legislation specific for the substance or mixture
No information available

16. OTHER INFORMATION

Full text of the R-phrases, hazard statements and precautionary statements mentioned in section 3:
H226 – Flammable liquid and vapour.
H302 – Harmful if swallowed.
H304 – May be fatal if swallowed and enters airways.
H315 – Causes skin irritation.
H317 – May cause an allergic skin reaction.
H400 – Very toxic to aquatic life.
H410 – Very toxic to aquatic life with long lasting effects.

SAFETY DATA SHEET (SDS)

Version 2.0
Revision Date : April 22,2022
Print Date : October 31, 2022

1. PRODUCT AND COMPANY IDENTIFICATION

Product name	:	Lovage Leaf Oil
Botanical name	:	Levisticum officinalis L.
Synonyms	:	Levisticum officinale
FEMA	:	2651
INCI name	:	Levisticum officinale Leaf Oil
CAS #	:	8016-31-7
Country of Origin	:	Hungary
EINECS #	:	84837-06-9
Product use	:	Domestic and Industrial
Supplier	:	New Directions Aromatics Inc.
Address	:	6781 Columbus Road, Mississauga, Ontario, CANADA L5T 2G9
Fax	:	905-362-1926
Telephone number	:	905-362-1915
Emergency phone number	:	(613)-996-6666 CANUTEC 24 HOUR EMERGENCY

2. HAZARDS IDENTIFICATION

Emergency Overview

WHMIS Classification

B3	Combustible Liquid.	
D2B	Toxic Material Causing	Moderate eye irritant
.	Other Toxic Effects	Moderate skin irritant

GHS Classification

Acute toxicity, Oral - Category 5

Skin Corrosion/Irritation - Category 2

Eye irritation - Category 2A.

Flammable Liquid Category 3

GHS Label elements, including precautionary statements

Signal: Warning

Hazard statement(s)

H315	Causes skin irritation.
H303	May be harmful if swallowed
H319	Causes serious eye damage.
H226	Flammable liquid and vapour.

Precautionary statement(s)

P305+P351+P338 .	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses if present and easy to do – continue rinsing.
P280	Wear protective gloves / protective clothing / eye protection / face protection.

3. COMPOSITION / INFORMATION INGREDIENTS

Product Name	CAS NO	EC NO	Concentration
N/A	-	-	-

4. FIRST AID MEASURES

Eye contact

Immediately flush eyes with plenty of cool water for at least 15 minutes. Get medical attention if irritation occurs.

Skin contact

Remove contaminated clothing. Wash area with soap and water. If irritation occurs, get medical attention.

Inhalation

If inhaled, removed to fresh air. Get medical attention if symptoms appear.

Ingestion

Seek medical attention or contact local poison control center.

5. FIRE FIGHTING MEASURES

Suitable extinguishing media

Foam. Dry Powder. Carbon dioxide.

Unsuitable extinguishing media

Water spray, water jet.

Special protective equipment and precautions for fire-fighters

Wear proper protective equipment. Exercise caution when fighting any chemical fire. Use water spray or fog for cooling exposed containers.

Special hazards arising from the substance or its combustible products

Hazardous decomposition products may be formed at extreme heat or if burned.

Resulting gases

Carbon oxides.

6. ACCIDENTAL RELEASE MEASURES

Personal precautions, protective equipment and emergency procedures.

Equip clean crew with proper protection. Respiratory protection equipment may be necessary.

Environmental precautions

Prevent entry to sewers and public waters. Notify authorities if product enters sewers or public waters.

Methods and materials for containment and cleaning up

Clean up any spills as soon as possible, using an absorbent material to collect it. Use suitable disposal

containers.

7. HANDLING AND STORAGE

Precautions for safe handling

No direct lighting. No smoking. Ensure prompt removal from eyes, skin and clothing. Wash hands and other exposed areas with mild soap and water before eating, drinking or smoking and when leaving work. Handle in accordance with good industrial hygiene and safety procedures.

Conditions for safe storage, including any incompatibilities

Provide local exhaust or general room ventilation to minimize dust and/or vapour concentrations. Keep container closed when not in use.

8. EXPOSURE CONTROLS AND PERSONAL PROTECTION

Eyes

Use tightly sealed goggles.

Skin

If skin contact or contamination of clothing is likely, protective clothing should be worn. Use protective gloves.

Respiratory

In case of insufficient ventilation, wear suitable respiratory equipment.

Ingestion

Handle in accordance with good industrial hygiene and safety practice. Wash hands before breaks and at the end of
workday.

9. PHYSICAL AND CHEMICAL PROPERTIES

Appearance	:	Pale yellow to amber liquid.
Odor	:	Warm spicy odor.
Flash point	:	60°C
Flammability(u/l)	:	
Relative density	:	0.900 - 0.970
Solubility (ies)	:	Soluble in alcohol.
Refractive index	:	1.470 - 1.495

10. STABILITY AND REACTIVITY

Reactivity

This material presents no significant reactivity hazard.

Chemical stability

Chemically stable.

Possibility of hazardous reactions

Hazardous polymerization will not occur.

Conditions to avoid

Avoid sparks, flame and other heat sources.

Incompatible materials

Strong oxidizing agents.

Hazardous decomposition products

Carbon Oxides.

11. TOXICOLOGICAL INFORMATION

Inhalation

Inhalation of high concentrations of vapor may result in irritation of eyes, nose and throat, headache, nausea, and dizziness.

Skin contact

Irritant to mucous membranes.

Eye contact

Possible irritation should be prevented by wearing safety glasses.

Serious eye damage

Causes serious eye damage.

12. ECOLOGICAL INFORMATION

Ecotoxicity

Avoid any pollution of ground, surface or underground water.

Persistence and degradability

Not available.

Bio - accumulative potential

Not available.

Mobility in soil

Not available.

Other adverse effects

Not available.

13. DISPOSAL CONSIDERATION

Dispose of product in accordance with local, state or provincial and federal regulations. Check with local municipal authority to ensure compliance.

14. TRANSPORT INFORMATION

UN Number

1169

UN proper shipping name

Extracts, aromatic, liquid

Transport hazard class

3

Packing group

III

US DOT Shipping Description (Land)

1169

Proper shipping name

Extracts, aromatic, liquid

Class

3

Packaging group

III

IMO-IMDG Shipping Description (Sea)

1169

Proper shipping name

Extracts, aromatic, liquid

Class

3

Packaging group

III

IATA Shipping Description (Air)

1169

Proper shipping name

Extracts, aromatic, liquid

Class

3

Packaging group

III

15. REGULATORY INFORMATION

WHMIS Classification

B3, D2B

GHS Hazard Statements

See section 2.

GHS Precautionary Statements

See section 2.

16. OTHER INFORMATION

Revision date : April 22,2022