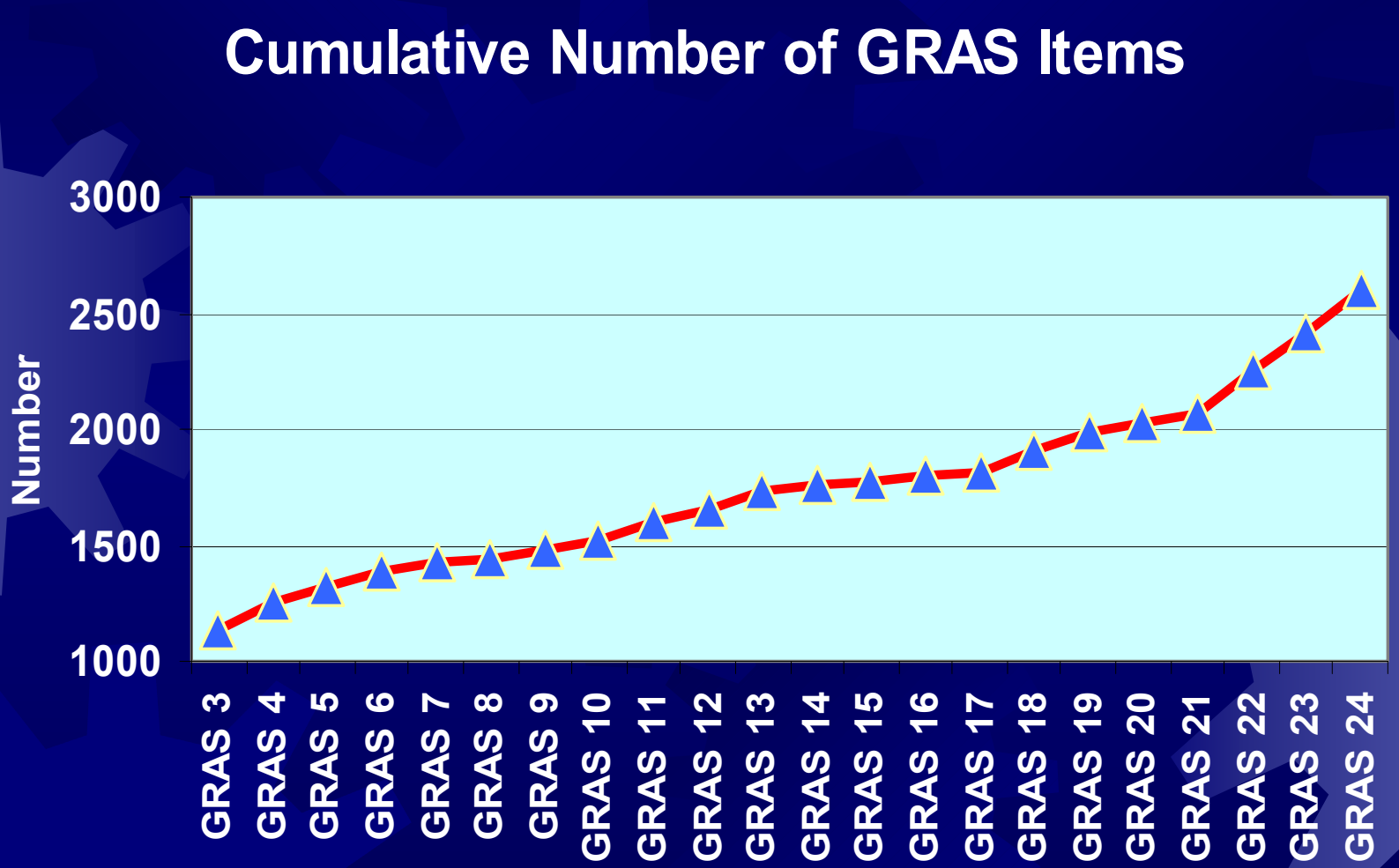
Odds & Trends in F&F – Technical Evolution & Information Revolution

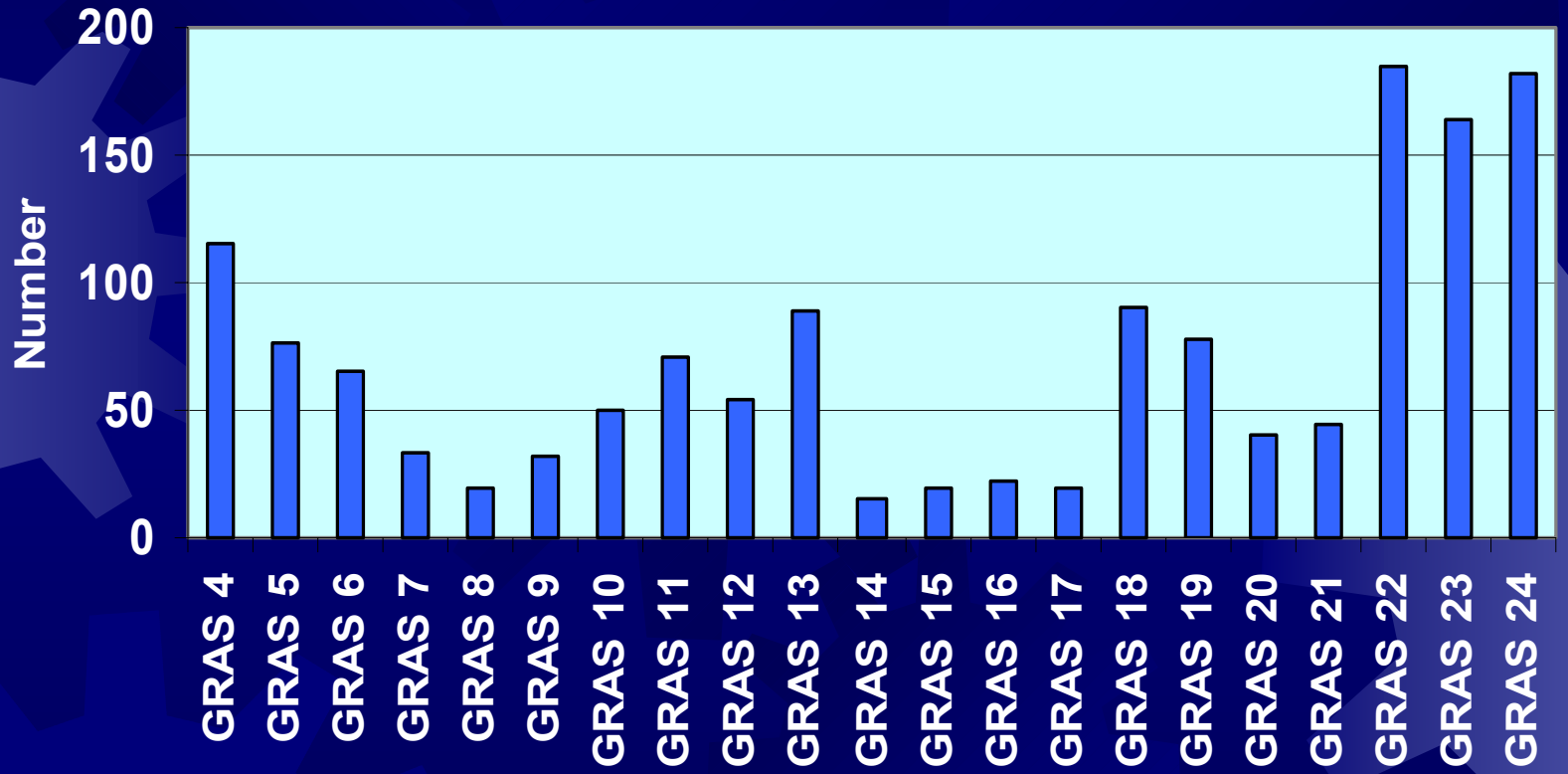
John C. Leffingwell
Society of Flavor Chemist's
November 20, 2008

Additions to Original GRAS List



Additions to Original GRAS List

Number of GRAS Items



20+ % from GRAS 22, 23 and the forthcoming GRAS 24

Technical Evolution of the F&F Industry

- 50's to 80's – Instrumentation - IR, NMR, GC-MS
- 90's to Present – Application of Drug design techniques, SAR, Biotechnology, Genomics
  - Today's Examples:
  - Receptor gene expression & ligand (agonist) screening for new potent tastants, flavor modifiers, cooling agents, sweetness enhancers, etc.
  - Methodology – Fluorescent Calcium Imaging (EC_{50}) to measure a ligands potency against a receptor

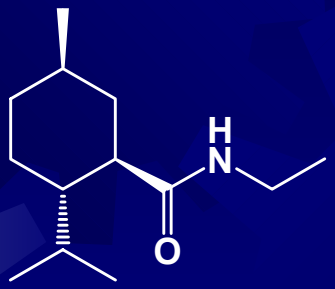
Thermo TRP Receptors & Agonists

Table 4 Thermoreceptor Agonists

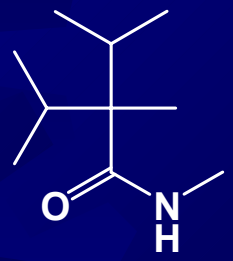
Chemical agonist (botanical source)	ThermoTRP
Capsaicin (hot chilli peppers, e.g., Tabasco)	TRPV1
Piperine (black pepper corns)	TRPV1
Allicin (fresh garlic)	TRPV1, TRPA1
Camphor (Cinnamomum camphora)	TRPV3, TRPV1
D-9-Tetrahydrocannabinol (Cannabis sativa)	TRPV2, TRPA1
2-Aminoethoxydiphenyl borate (synthetic)	TRPV1, TRPV2, TRPV3
4-a-phorbol 12,13-didecanoate (synthetic)	TRPV4
(-)-Menthol (peppermint)	TRMP8, TRPV3
1,8-Cineole, eucalyptol (eucalyptus)	TRPM8
WS-3 (synthetic)	TRPM8
Icilin (synthetic)	TRPM8, TRPA1
Cinnamaldehyde (cinnamon, cassia)	TRPA1, TRPV3
Allyl isothiocyanate (mustard, horseradish)	TRPA1
Benzyl isothiocyanate (mustard, horseradish)	TRPA1
Phenethyl isothiocyanate (mustard, horseradish)	TRPA1

Leffingwell, Handbook of Cosmetic Science and Technology, 3d edition (2009), In Press

Amide GRAS Cooling Agents Thru GRAS 23

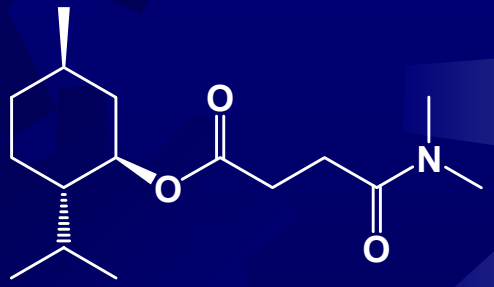


WS-3
FEMA 3455
1.5 X Menthol



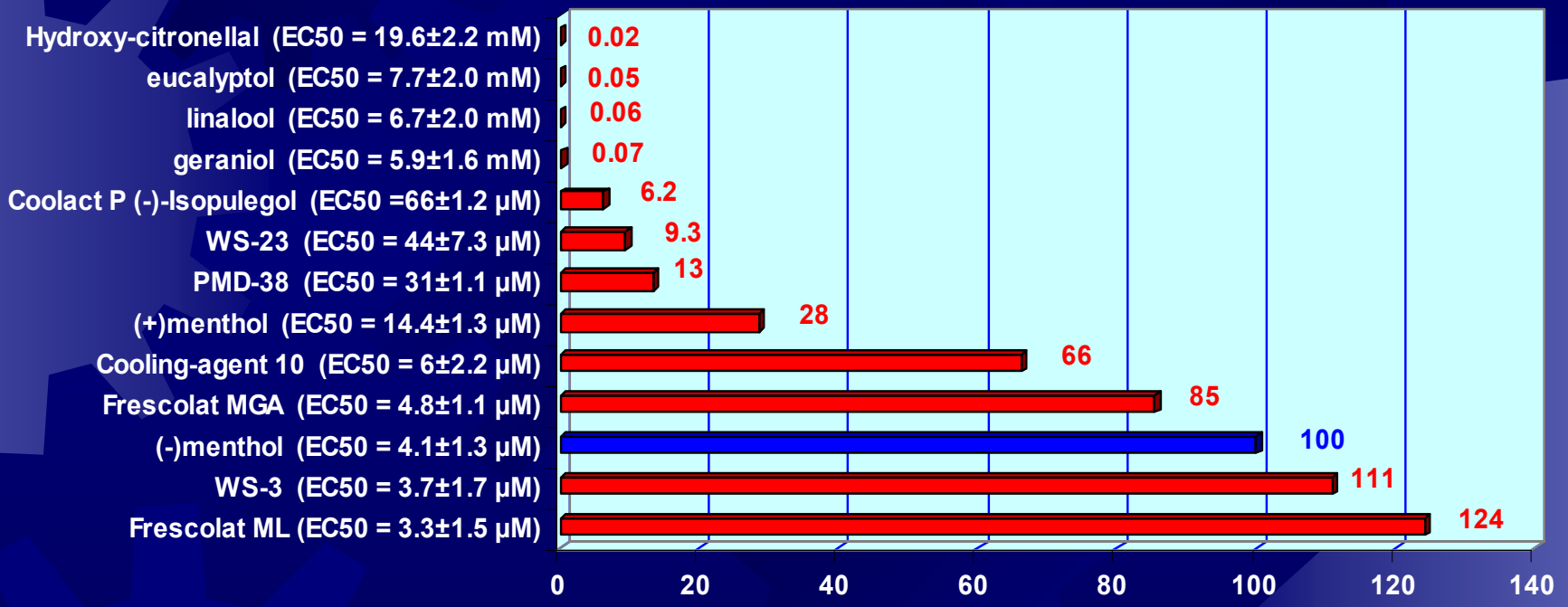
WS-23
FEMA 3804
0.75 X Menthol

WS-5
FEMA 4309
4 X Menthol



Menthyl N,N-dimethylsuccinamide
FEMA 4230

Relative Potency of TRPM8 agonists based on EC_{50} values (mean) with (-)-Menthol = 100



Note - Icilin (EC_{50} =0.2±0.1 µM) not shown
(Relative icilin potency vs (-)-Menthol at 100 is 2050) - Adapted from Behrendt, et .al, Brit. J. Pharm., 141:737-745 (2004)

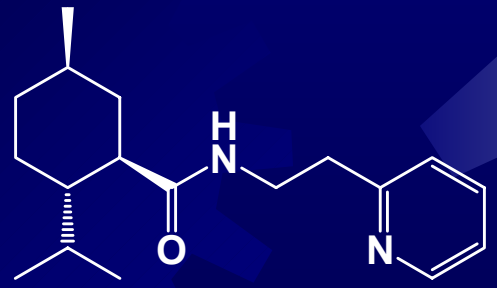
Givaudan's New Powerful GRAS Cooling Agents

Table 1 Comparison of data for the TRPM8 activation, expressed as EC_{50} and isointensity to menthol of selected menthane carboxamides

Cpd.	R_1	R_2	EC_{50} (μM)	Isointensity (ppm)	CAS no.
2a	CH_3	CH_3	25.6	2.0	39668-77-4
2b	C_2H_5	H	3.6	1.5	68489-00-9
2c	$CH_2C_6H_5$	H	3.5	2.0	73435-72-0
2d	4-$C_6H_4SO_2NH_2$	H	0.7	0.5	852379-29-4
2e	C_6H_5	H	0.6	1.0	824947-52-6
2f	2-$C_6H_4CONH_2$	H	0.6	0.5	915962-26-4
2g	4-$C_6H_4OCH_3$	H	0.5	2.0	68489-09-8
2h	4-$C_6H_4CONH_2$	H	0.4	0.15	847564-94-7
2i	4-$C_6H_4CH_2OH$	H	0.4	1.0	847564-90-3
2j	4-$C_6H_4CH_2CN$	H	0.2	0.2	852379-28-3
2k	CH_2CH_2 (2-C_5H_4N)	H	0.1	0.05	926913-58-8
2l	2-$C_6H_5OCH_3$	H	5.1	2.0	824947-60-6
2m	$CH_2CH_2CH_2$ $OCH(CH_3)_2$	H	0.9	0.7	663218-92-6

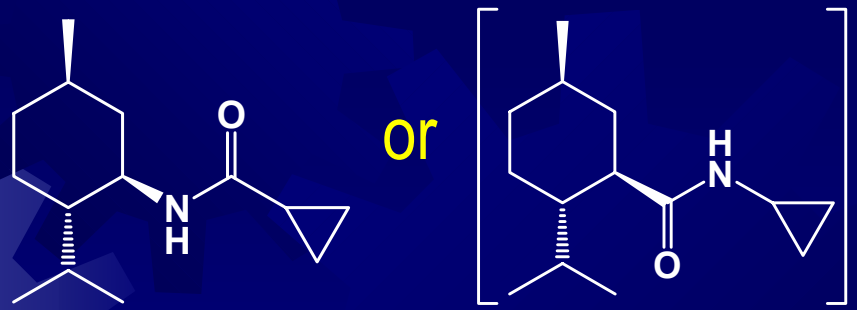
Furrer et al., Chem. Percept. (July 2008) 1:119–126; Galopin et al., US Patent 7,414,152 (August 19, 2008); Bell et al., WO2007019719 (Feb. 22, 2007)

2j = FEMA 4496
10X Menthol

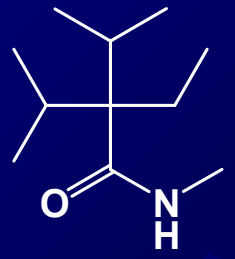


2k = FEMA 4549
40-100X Menthol

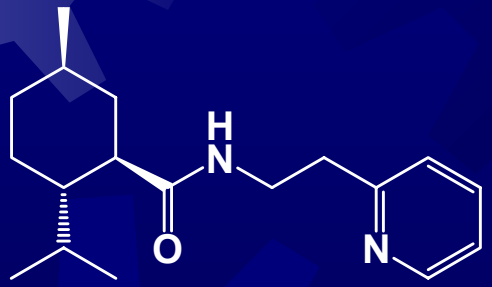
New GRAS 24 Cooling Agents



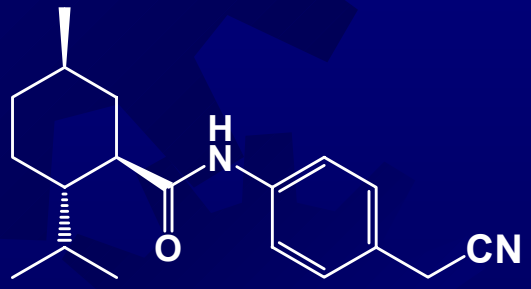
Menthyl cyclopropanecarboxamide
FEMA 4558



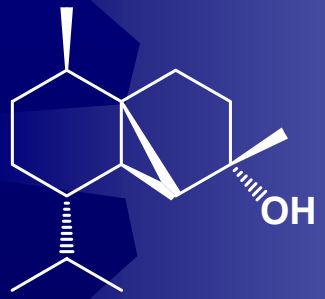
N-Ethyl-2,2-diisopropyl-butanamide
FEMA 4557



N-(2-(Pyridin-2-yl)ethyl)-3-p-menthanecarboxamide
FEMA 4549
40-100 X Menthol

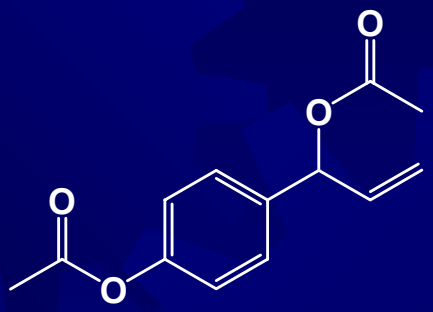


N-p-Benzeneacetonitrile-menthanecarboxamide
FEMA 4496
10 X Menthol

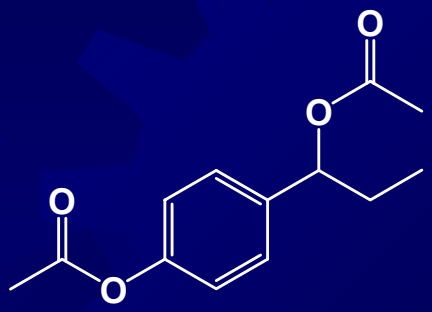


Cubebol
FEMA 4497

New GRAS Warming / Pungent Agents



Galangal acetate
Not GRAS but Galangal extracts are
Yang & Eilerman, J. Agric. Food Chem. 1999, 47, 1657-1662

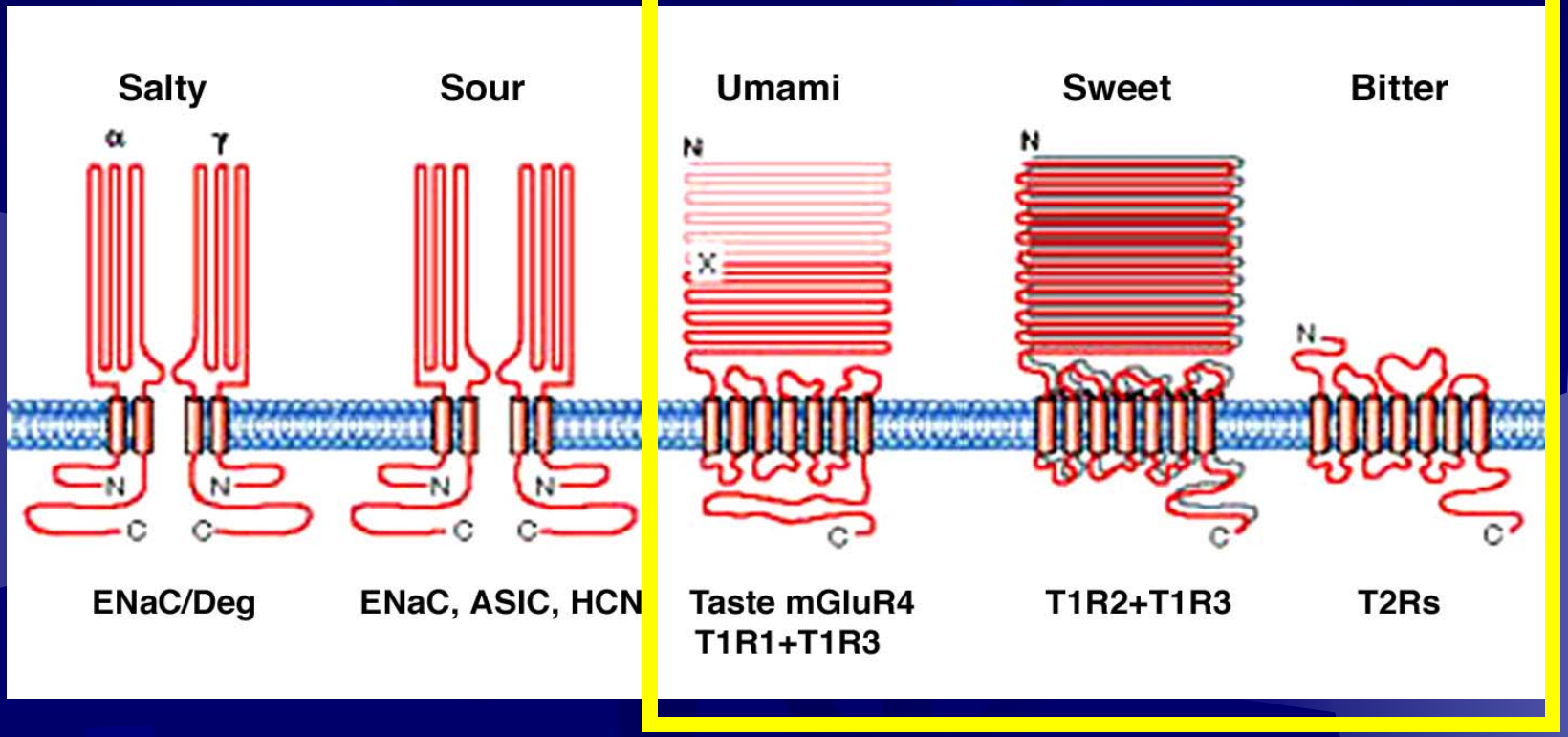


Dihydrogalangal acetate
FEMA 4555
Bachmann et al. EP1700525

Wasabi-like GRAS 23 Warming / Pungent Agents

Erucin – FEMA 4414
Lesquerellin – FEMA 4415
Berteroin – FEMA 4416
Amyl isothiocyanate – FEMA 4417
3-Butenyl isothiocyanate – FEMA 4418
sec-Butyl isothiocyanate – FEMA 4419
Ethyl isothiocyanate – FEMA 4420
5-Hexenyl isothiocyanate – FEMA 4421
Hexyl isothiocyanate – FEMA 4422
Isoamyl isothiocyanate – FEMA 4423
Isobutyl isothiocyanate – FEMA 4424
Isopropyl isothiocyanate – FEMA 4425
Methyl isothiocyanate – FEMA 4426
4-pentenyl isothiocyanate – FEMA 4427
Benzyl isothiocyanate – FEMA 4428

Taste Receptors



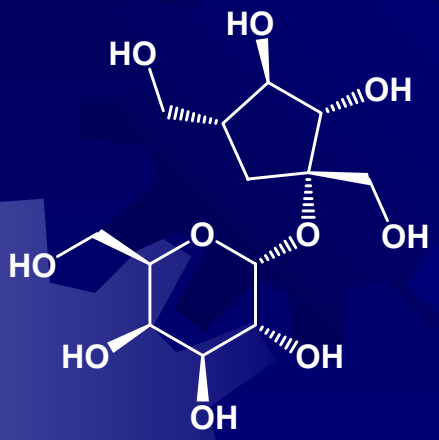
Lindemann, *Nature* 413: 219-225 (2001)

Salt & Sour Receptors are Ion Channels

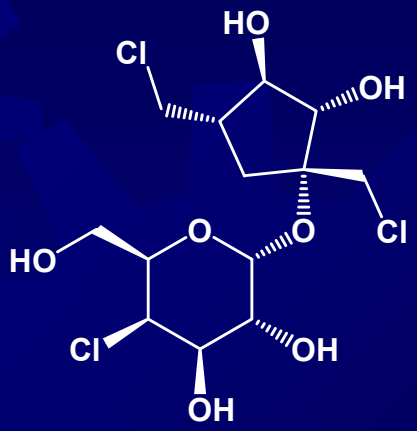
Umami, Sweet & Bitter Receptors are GPCR Receptors

For examples (sweet and umami) - See Li et al., *PNAS, 2002, 99 (7) 4692-4696*

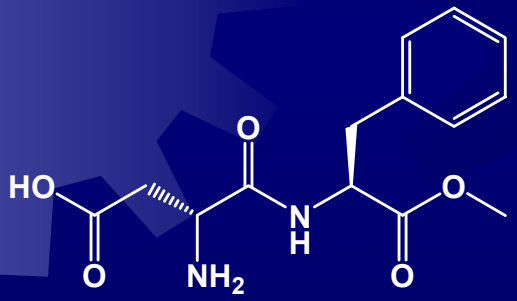
Diverse Compounds are Sweet



Sucrose



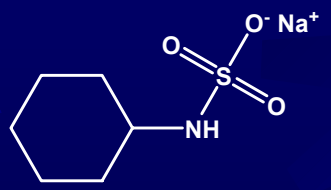
Sucralose



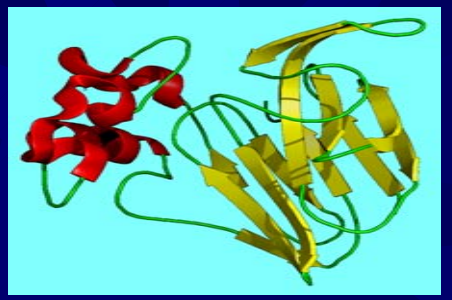
Aspartame

Acesulfame K

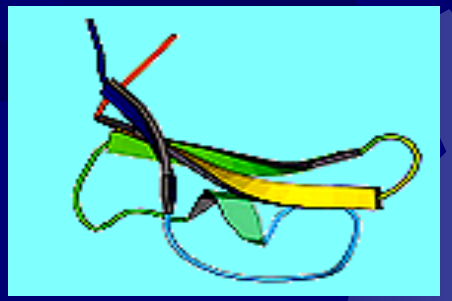
Saccharin



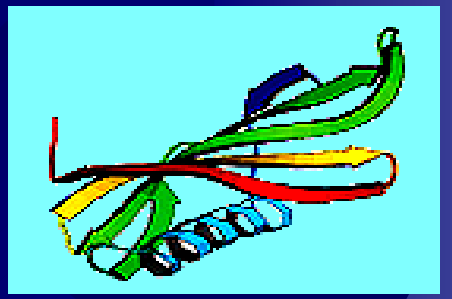
Cyclamate



Thaumatin

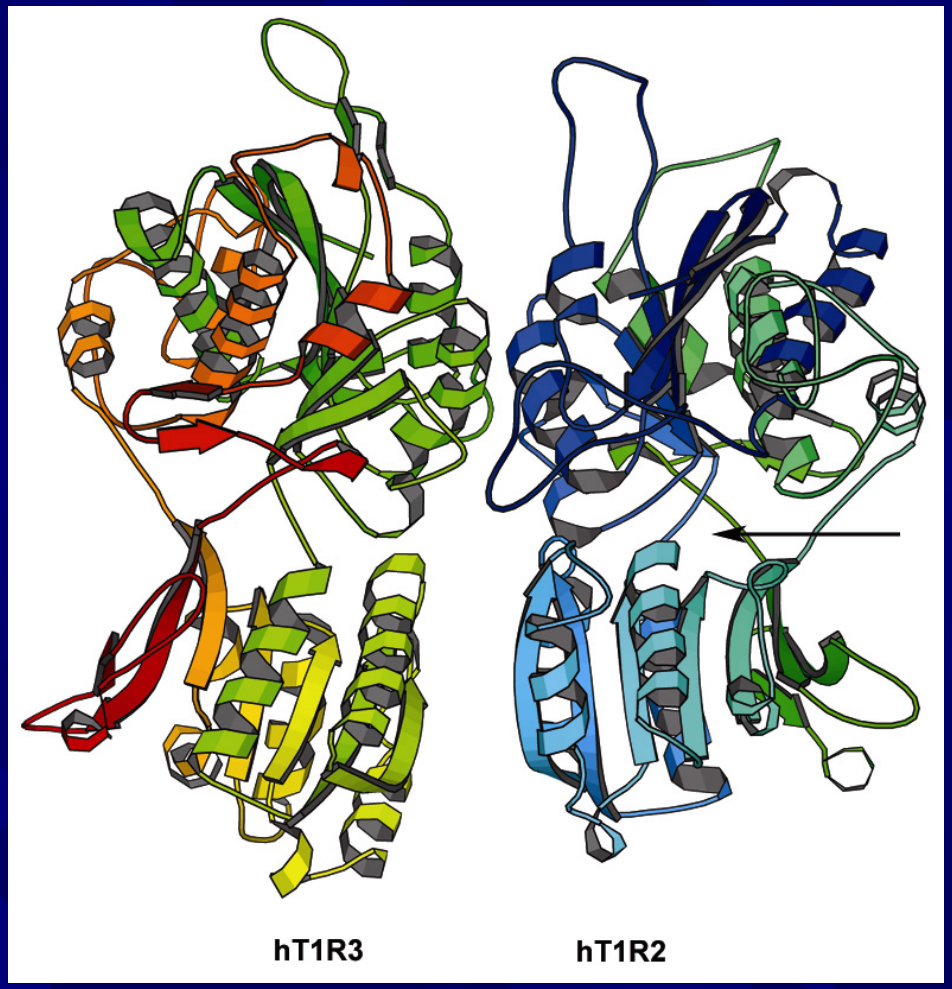


Brazzein



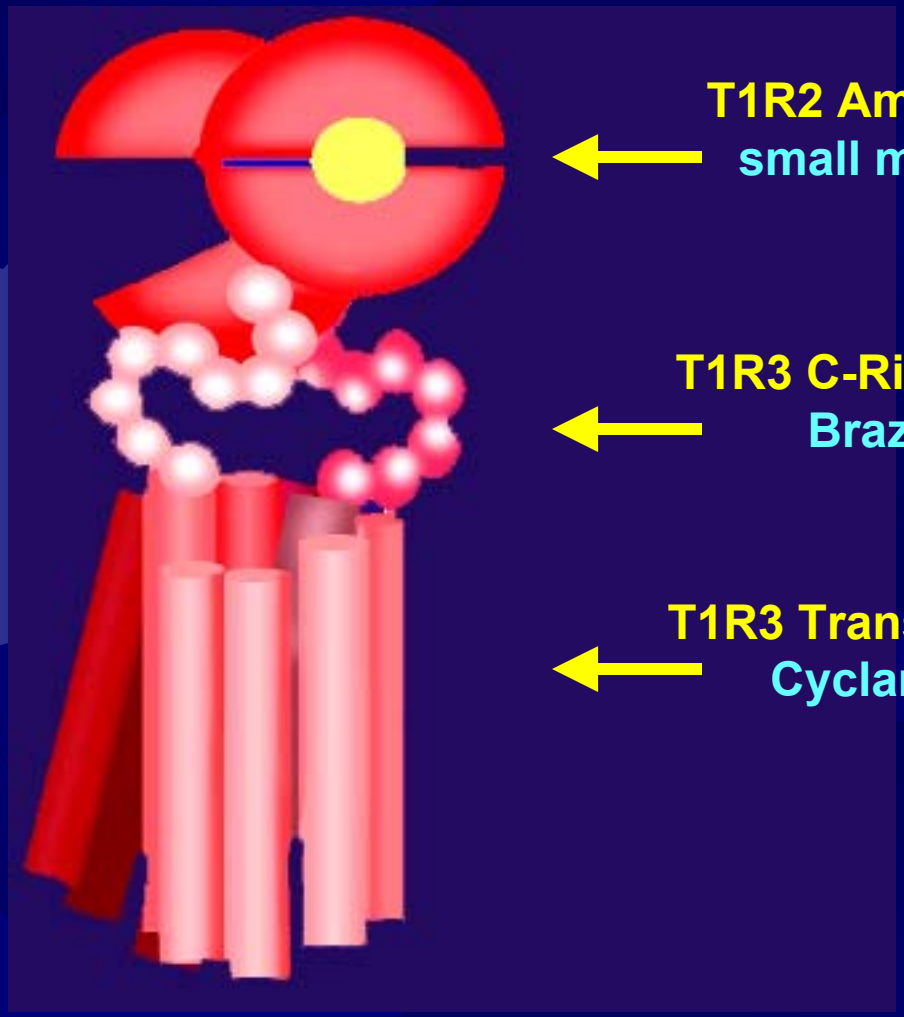
Monellin

Multiple Regions of T1R2+T1R3 are Required for Effective Interaction with Different Sweet Compounds



R. F. Margolskee, *Sci. STKE*, 28 June 2005

Multiple Regions of T1R2+T1R3 are Required for Effective Interaction with Different Sweet Compounds



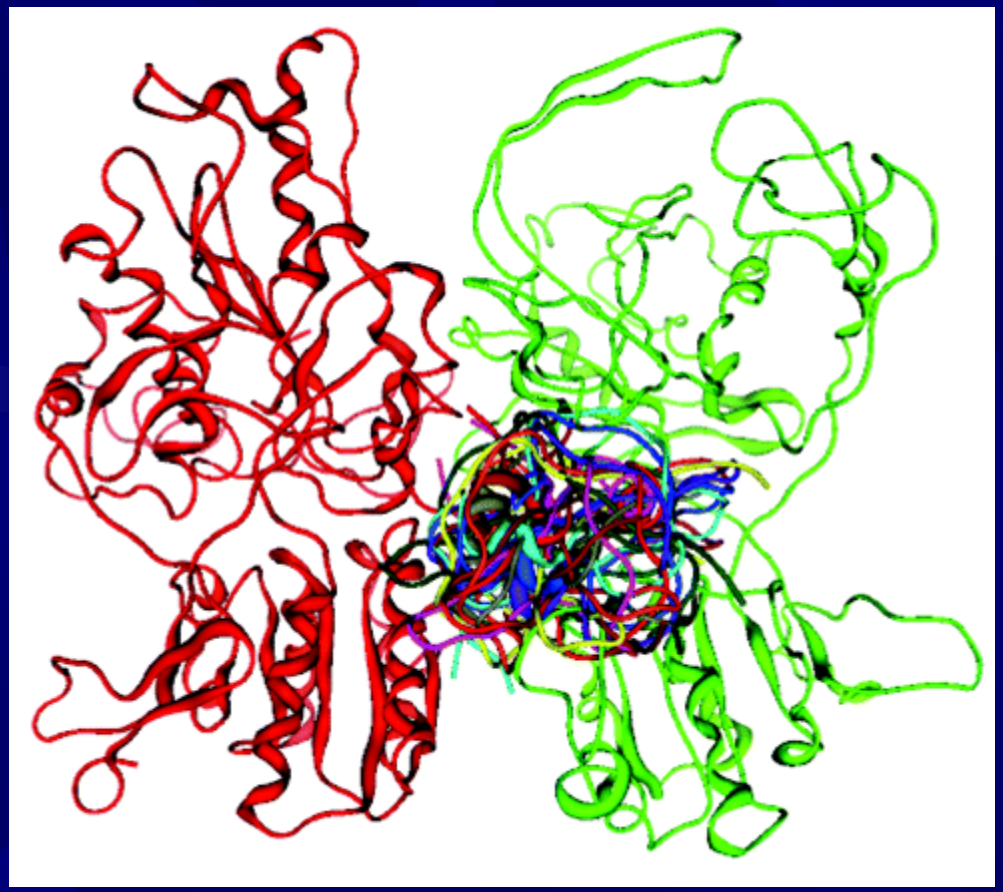
T1R2 Amino Terminal Domain
small molecule sweeteners

T1R3 C-Rich Region
Brazzein

T1R3 Transmembrane Region
Cyclamate, Lactisole

R. F. Margolskee, *Sci. STKE*, 28 June 2005

Sweet Protein Brazzein Docked to the T1R3 Subunit



Walters et al., J Agric Food Chem. 2006; 54(26): 10129–10133

Synomyx Sweetness Enhancers

November 5, 2008 – Senomyx receives GRAS status for S2383 – an enhancer that allows a 75% reduction of Sucralose

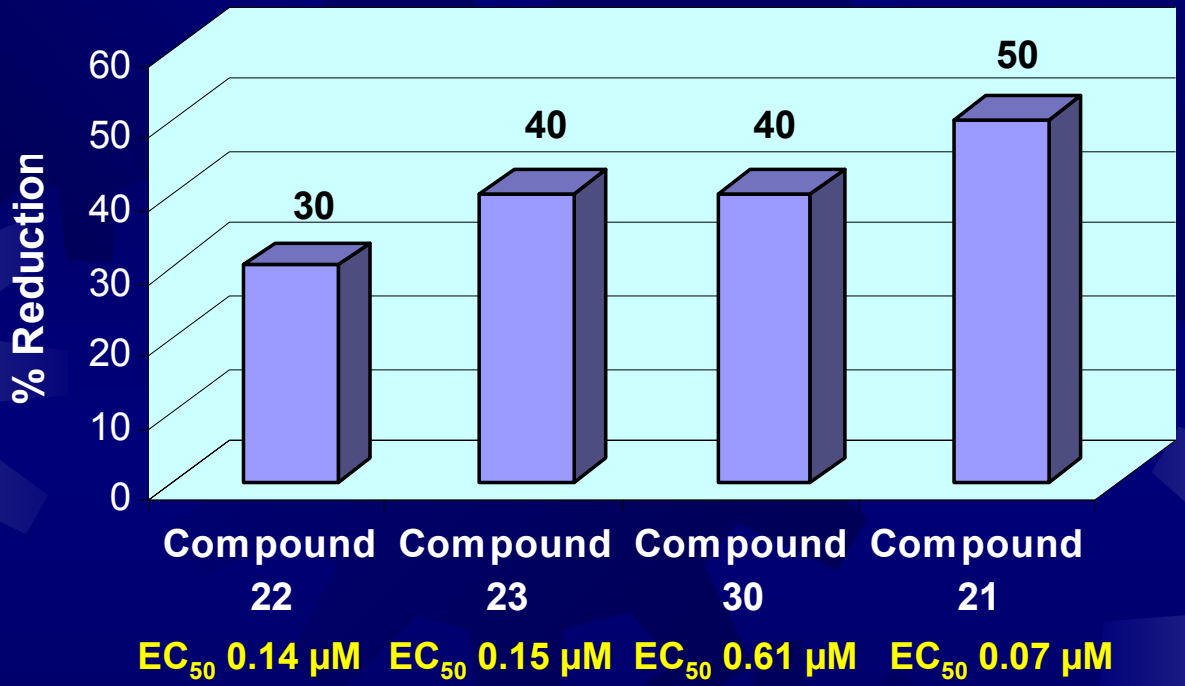
November 6, 2008 – Senomyx enters a collaborative commercialization and license agreement with Firmenich for S2383

Senomyx has also initiated development activities for S6973, a new sucrose enhancer that enables up to a 50% reduction of sucrose in taste tests with beverage, yogurt, and other product prototypes

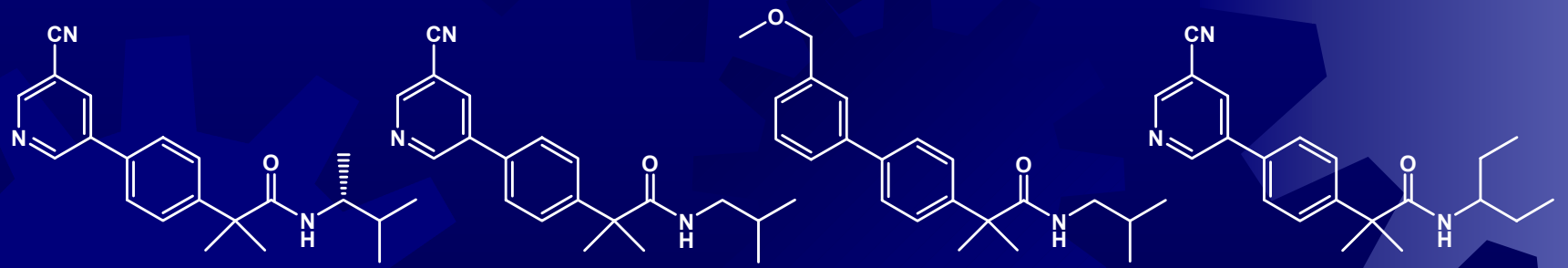
What are S2383 & S6973 = ?? At the moment, but…

Synomyx Sweetness Enhancers

% Fructose / Glucose Reduction



EC_{50} 0.14 µM EC_{50} 0.15 µM EC_{50} 0.61 µM EC_{50} 0.07 µM

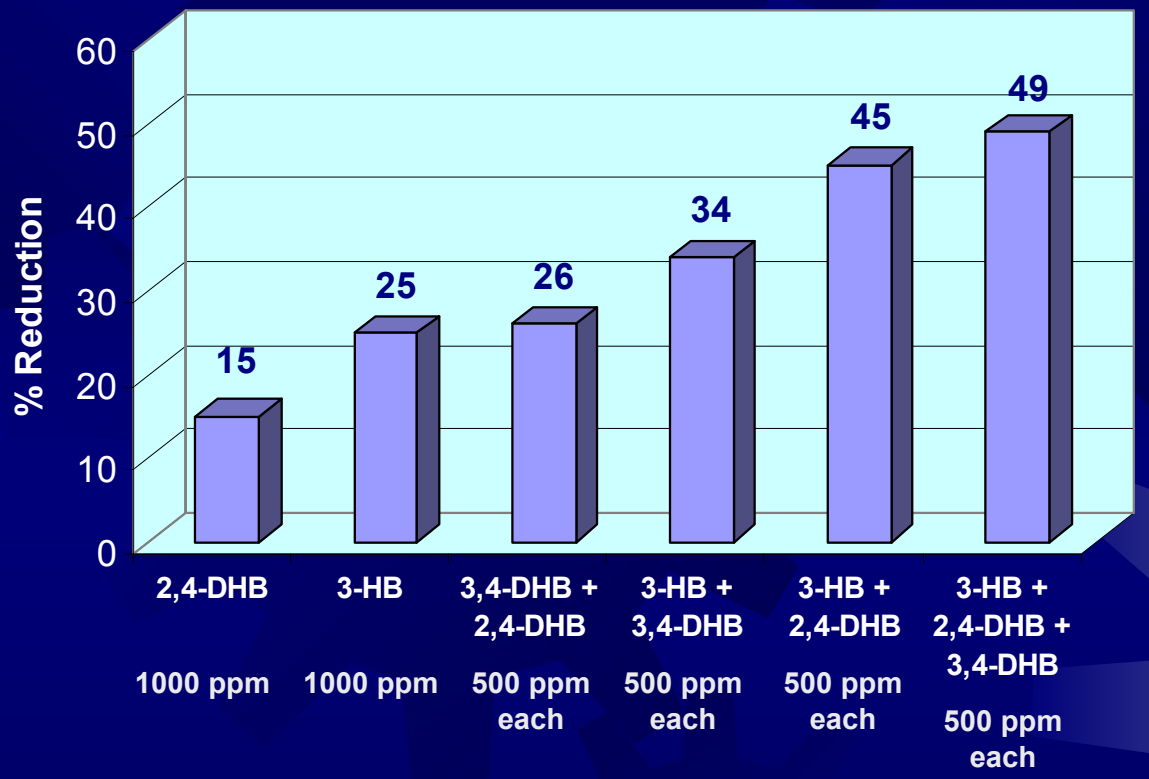


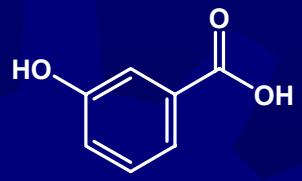
Compound 22 Compound 23 Compound 30 Compound 21

Tachdjian et al.. US Patent Application 20070003680 (2007)

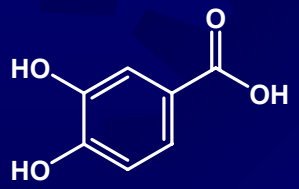
3-Hydroxy & Dihydroxybenzoic acids as Sweetness Enhancers

Effect of Blend Ratios on Sucrose Reduction

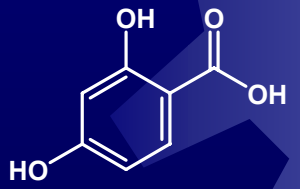




3-Hydroxybenzoic acid
FEMA 4431



3,4-Dihydroxybenzoic acid
FEMA 4430



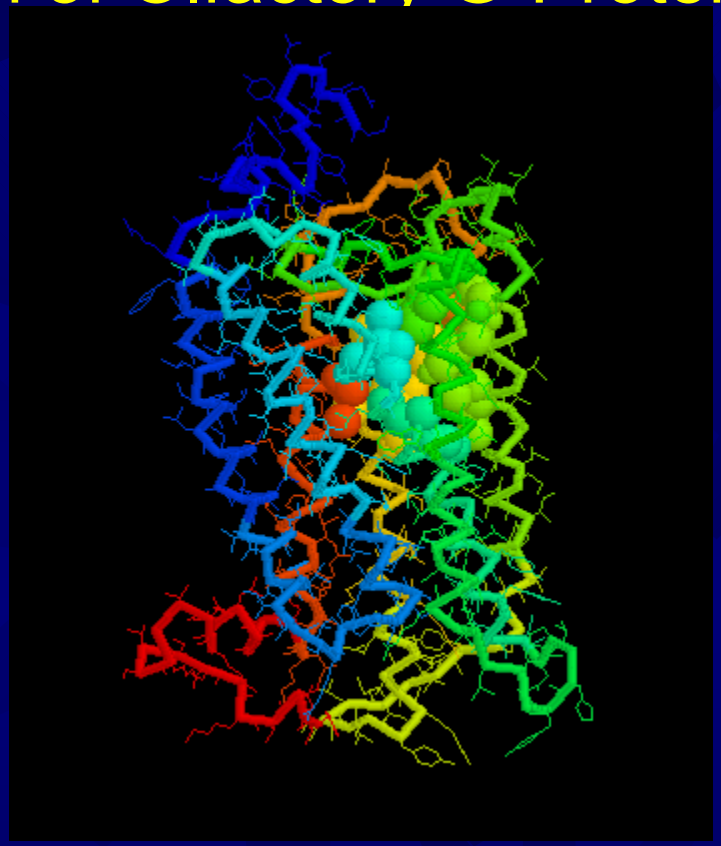
2,4-Dihydroxybenzoic acid
FEMA 3798

Bingley et al, US Patent Application 20070054023 (2007) to Cadbury

New GRAS Bitter Blocker

cis-4-(2,2,3-Trimethylcyclopentyl)butanoic acid
FEMA 4529
Ungureanu et al., PCT WO2008119197 (Oct. 9, 2008)

Elucidation of Olfactory G-Protein Receptors

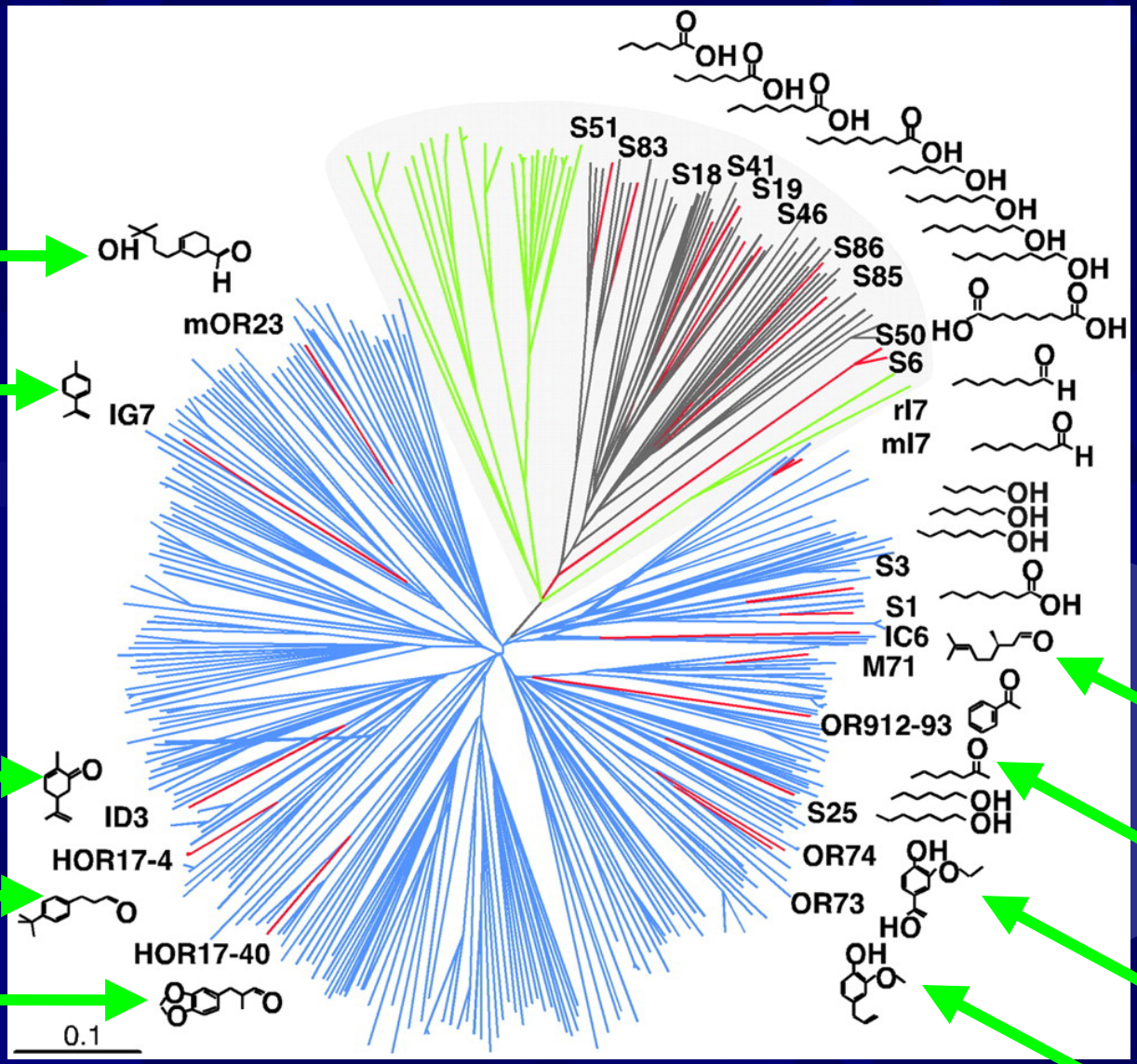


Putative Binding cavity in Human OR1.04.06

900+ Human Olfactory Receptor Genes Identified – D. Lancet
~600 Pseudogenes + ~300 Intact Genes

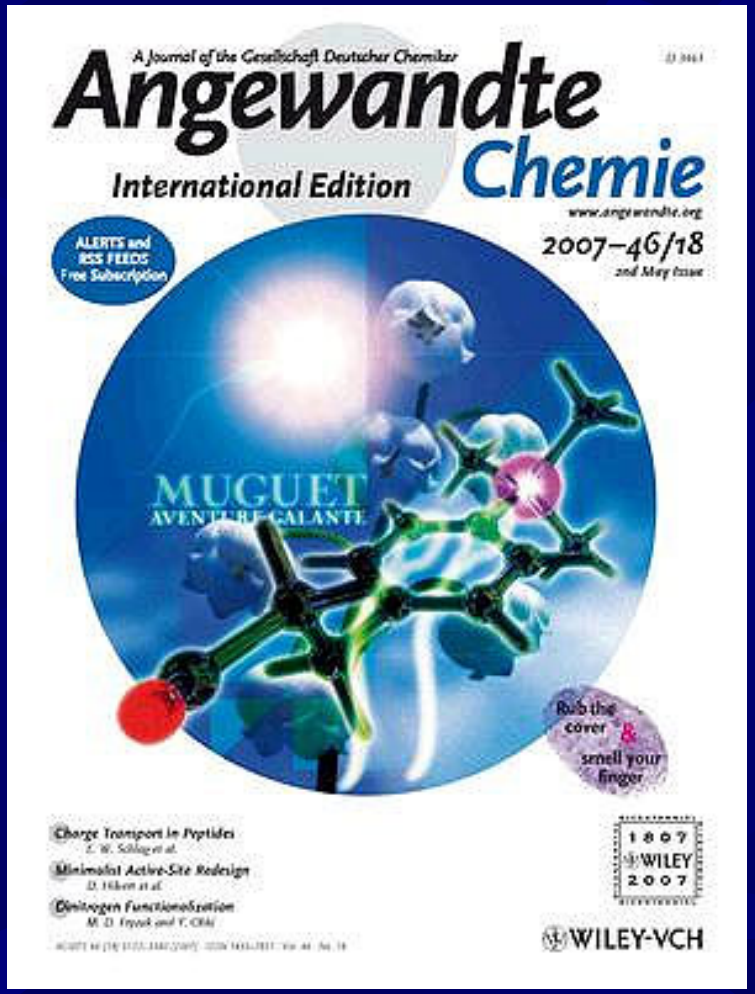
Glusman, Yanai, Rubin, Lancet, Genome Res. 2001 May;11(5):685-702; Zozulya, Echeverri, Nguyen, Genome Biology 2001, 2(6):research0018.1–0018.12; Malnic, Godfrey, Buck, PNAS, 2004, 101 (8), 2584–2589

Phylogenetic tree of sequence relationships among ORs



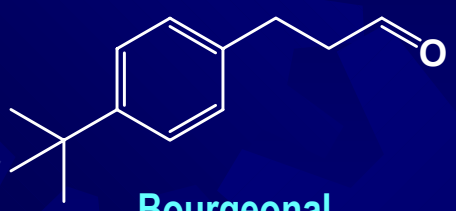
Malnic, Godfrey, Buck, PNAS, 2004, 101 (8), 2584–2589

Prediction of Perception: Probing the hOR17-4 Olfactory Receptor Model with Silicon Analogues of Bourgeonal and Lilial

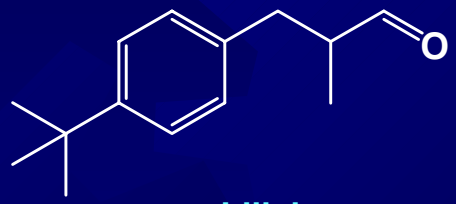


L. Doszczak, P. Kraft, et al., Angew. Chemie, 46 (18), 2007, 3367-3371

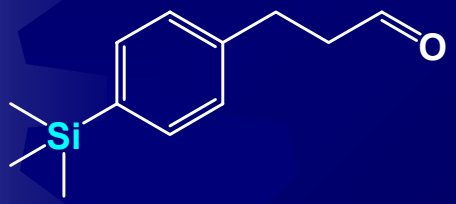
Prediction of Perception: Probing the hOR17-4 Olfactory Receptor Model with Silicon Analogues of Bourgeonal and Lilial

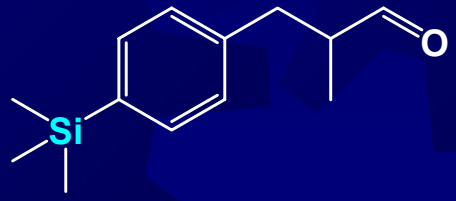


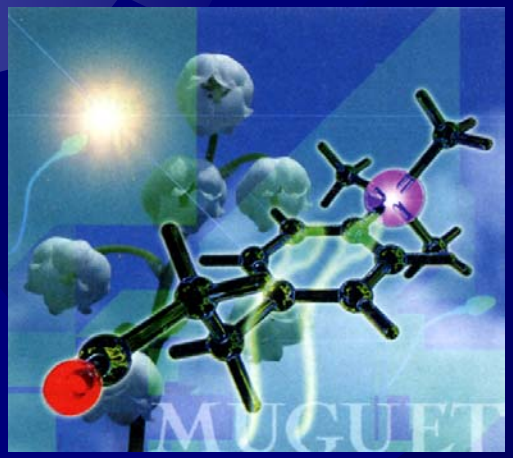
Bourgeonal



Lilial



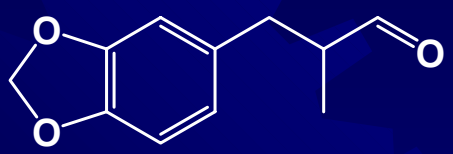




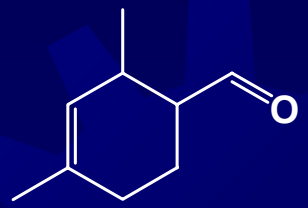
Sense and sensibility: Silicon analogues of the lily-of-the-valley odorants Lilial and Bourgeonal demonstrate that the electronic surface structure determines the interaction of an odorant with its.olfactory receptor. The subtle changes in the stereoelectronic properties enable a comparison of in vivo, in vitro, and in silico data. Odor thresholds, as well as the swimming behavior of sperm cells, correlate well with the binding energies obtained from a computational model of the hORI7-4 receptor.

L. Doszczak, P. Kraft, et al., Angew. Chemie, 46 (18), 2007, 3367-3371

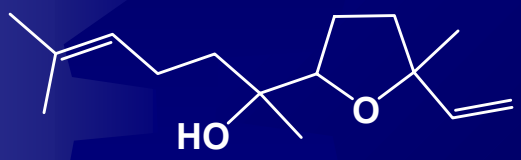
Fragrance Materials - Newly GRAS



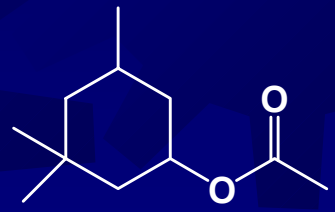
Helional®
FEMA 4599
Floral, green, ozone, marine



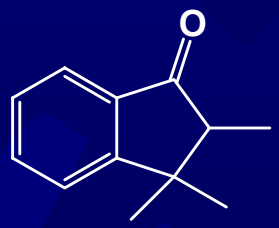
Triplal®, Cyclal C, Vertocitral
FEMA 4505
Green, Citrus, Herbal, Aldehydic



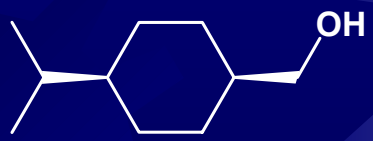
Nerolidol oxide
FEMA 4536
Strawberry- and milk-like fruity and floral fragrance; red berry flavor



3,3,5-Trimethylcyclohexyl acetate
FEMA 4512
Brightly fresh-fruity, minty, herb-like and floral-roselike



Safraleine®
FEMA 4556
Leathery, spicy, warm, woody odor, reminiscent of saffron



Mayol®
FEMA 4507
Fresh floral odor reminiscent of many flowers - magnolia, tuberose, muguet

The F&F Information Revolution

- 50's to 90's –
  - Patent Literature – Chemical Abstracts & order a copy (may take months)
  - Bedoukian's reviews (once a year)
  - Numerous periodicals (e.g. Helv. Chim. Acta, J. Org. Chem., J. Agric. Food Chem. etc.)
  - Technical Books (Guenther, Arctander, Bedoukian)
  - All time consuming
- Mid 90's to Present –
  - Patents & Patent Applications online (searchable)
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  - Industry web sites (with lots of free Info)
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Patents

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    Has a Japanese to English Patent translator**

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    Searches any and all ACS journals - (abstracts free)
  - Wiley InterScience - http://www3.interscience.wiley.com/search/allsearch
    Searches many journals of F&F interest
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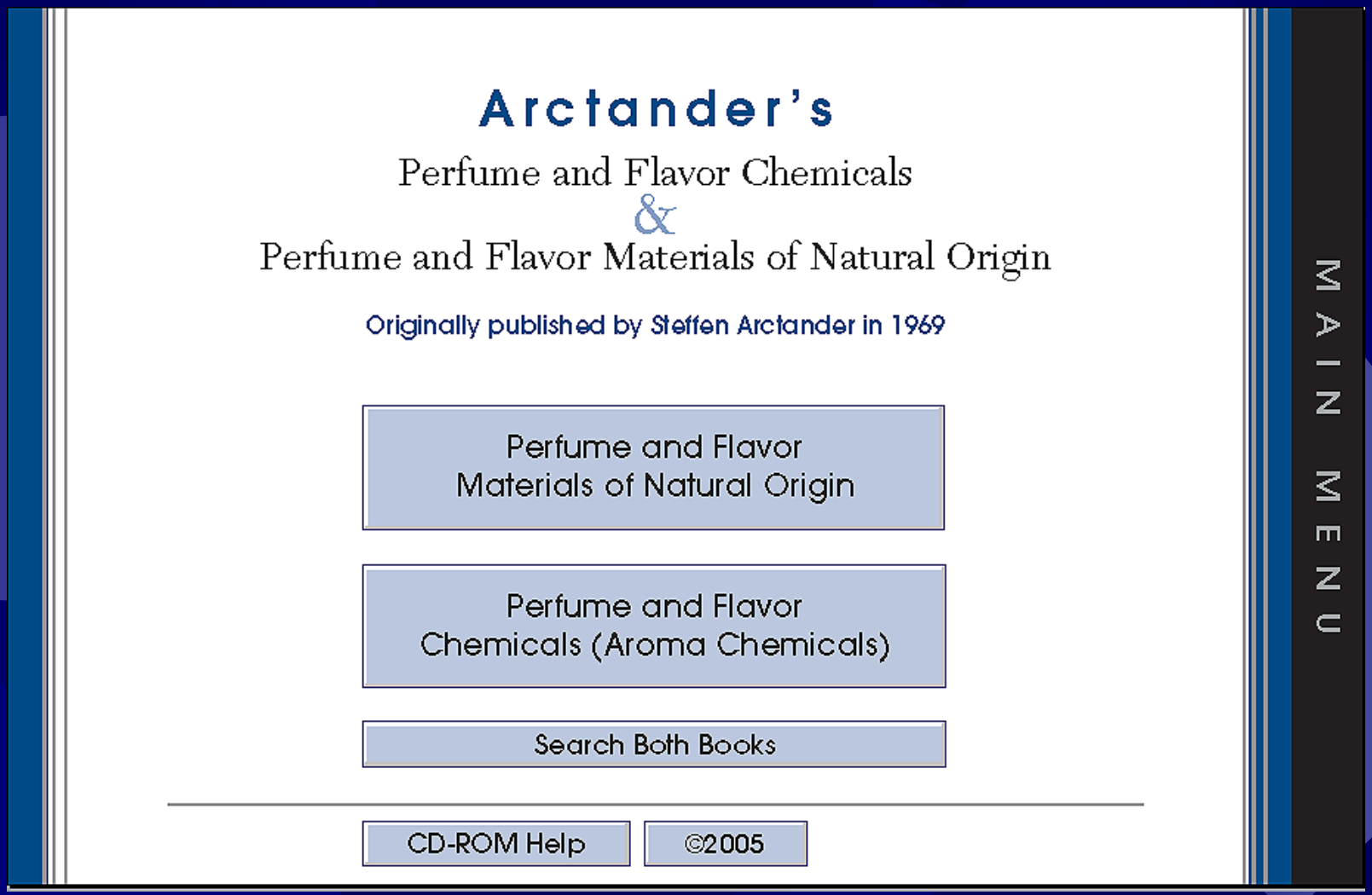
Industry web sites

- Perfumer & Flavorist – http://www.perfumerflavorist.com/ - great site for industry news; can purchase P&F magazine and J. Essential Oil Res. Articles; Mosciano's database; Bookstore
- Leffingwell & Associates – http://www.leffingwell.com/ - industry news (e.g. Top 10 F&F); lots of technical information & links (all free)
- JECFA – http://www.fao.org/ag/agn/jecfa-flav/search.html - Search JECFA status and specs
- Japanese food & flavor additives - http://www.ffcr.or.jp/
- EU flavor database – all Flavis numbers http://ec.europa.eu/food/food/chemicalsafety/flavouring/database/dsp_search.cfm
- Others – EFSA, FKS, IOFI, FEMA, Good Scents Co.

Books & P&F on CD-ROM



Books & P&F on CD-ROM

Leading F&F Software

Leffingwell & BACIS Databases

- Flavor- Base 2007
- ESO 2006 – Database of Essential Oils
- VCF 2000 – Volatile Compounds in Foods
- PMP 2001 – Database of Perfumery Materials
- FRM 2001 – Database of Flavor Raw Materials
- PFC 2002 – Perfume & Fragrance Classifications

Beverage & Juice Formulation Software

- Beverage-Master 2004
- Juice-Master 2004

Flavor-Base 2007

FLAVOR-BASE PRO "2007"

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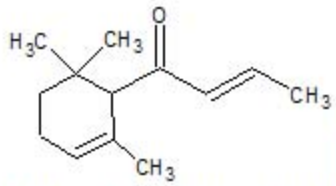

Molecular formula = C 13 H2
Molecular Weight = 192.29
Composition = C(81.2
Molar Refractivity = 59.82 ±
Molar Volume = 214.0 ±
Parachor = 492.0
Index of Refraction = 1.471 ±
Surface Tension = 27.9 ±
Density = 0.898
Polarizability = 23.71 ±

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GRAS & EC FLAVOR CHEMICALS

Rec. # 500

Name: Damascone; alpha-Damascone;

Synonyms: 4-(2,6,6-Trimethyl-2-cyclohexenyl)-2-butene-4-one;

Suppliers: Firmenich, Moellhausen

Flavor Description: Fruity (apple-citrus), tea-like with a slight minty note

Natural Occurrence: Tea.

Comments: Odor threshold in water: 1.5 ppb for the (+)-alpha-damascone and 100 ppb for the (-)-alpha-damascone (Pickenhagen, 1989). Mosciano, et. al. report that this material has a "sweet, fruity, floral woody with a green berry nuance" odor and a "sweet, fruity, woody with a green seedy background" taste at 30 ppm. alpha-Damascone is very expensive, but does find some utility in Tea, Tobacco and as a modifier in a few Fruit flavors. Except for Tea type applications, the author finds both beta-damascone

Image | EC Register | Japan Flavor Chemicals

FEMA No: 3659
COE No. & Cat.: 11053
FDA No:
EC Register: 07.134
JECFA No. 385 Japan: X
CAS No: 43052-87-5
Sp. Gravity: 0.898
Flash Point: >212 F. >100 C.
EINECS: 256-430-4
Nature: Nature Identical
C13 H20 O
Mol. Wt.: 192.297
Sol in Water:
Sol in Ethanol: SOL
Sol in P.G: SOL
Sol in Oil: SOL

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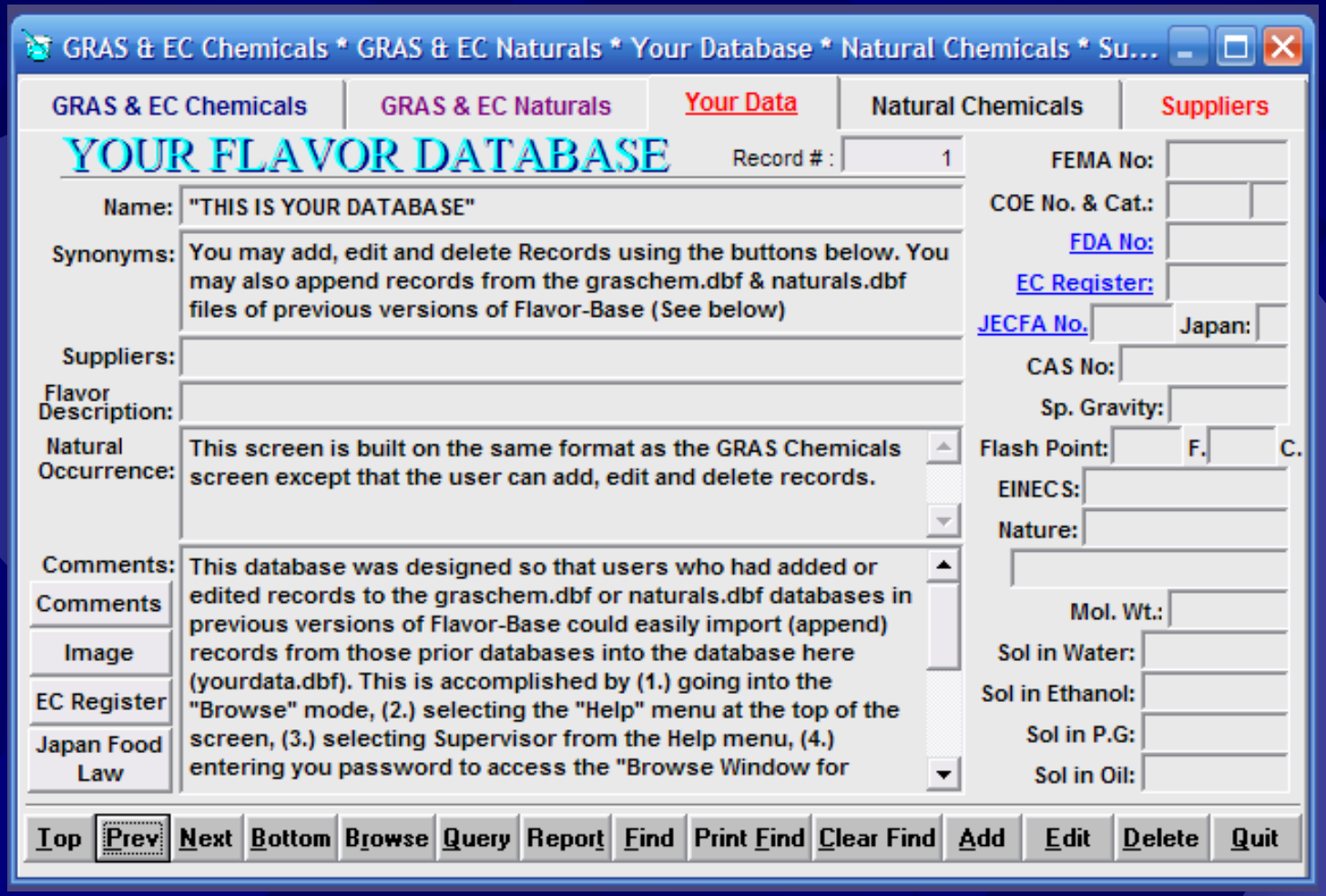
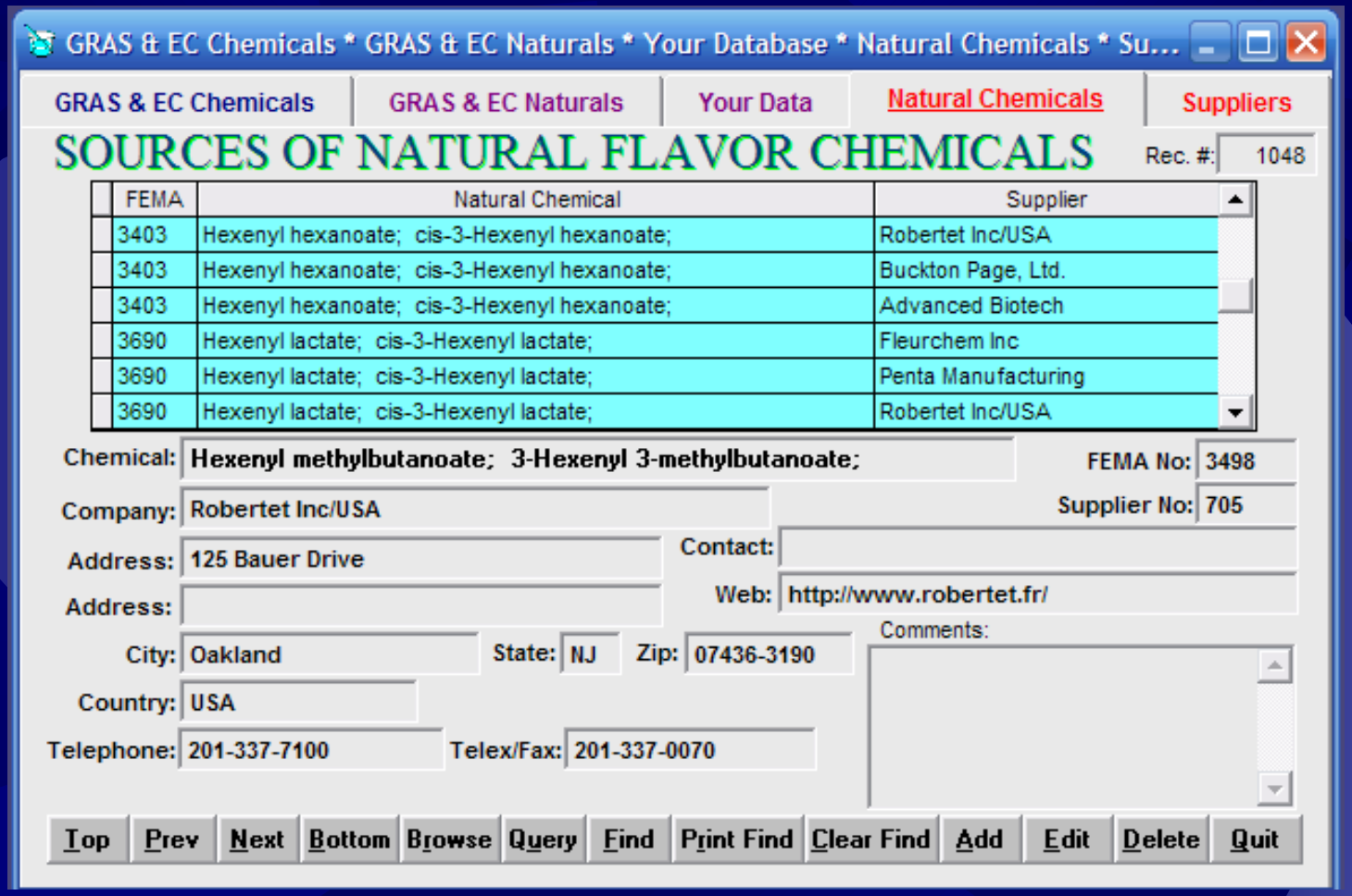
Flavor-Base 2007



Flavor-Base 2007



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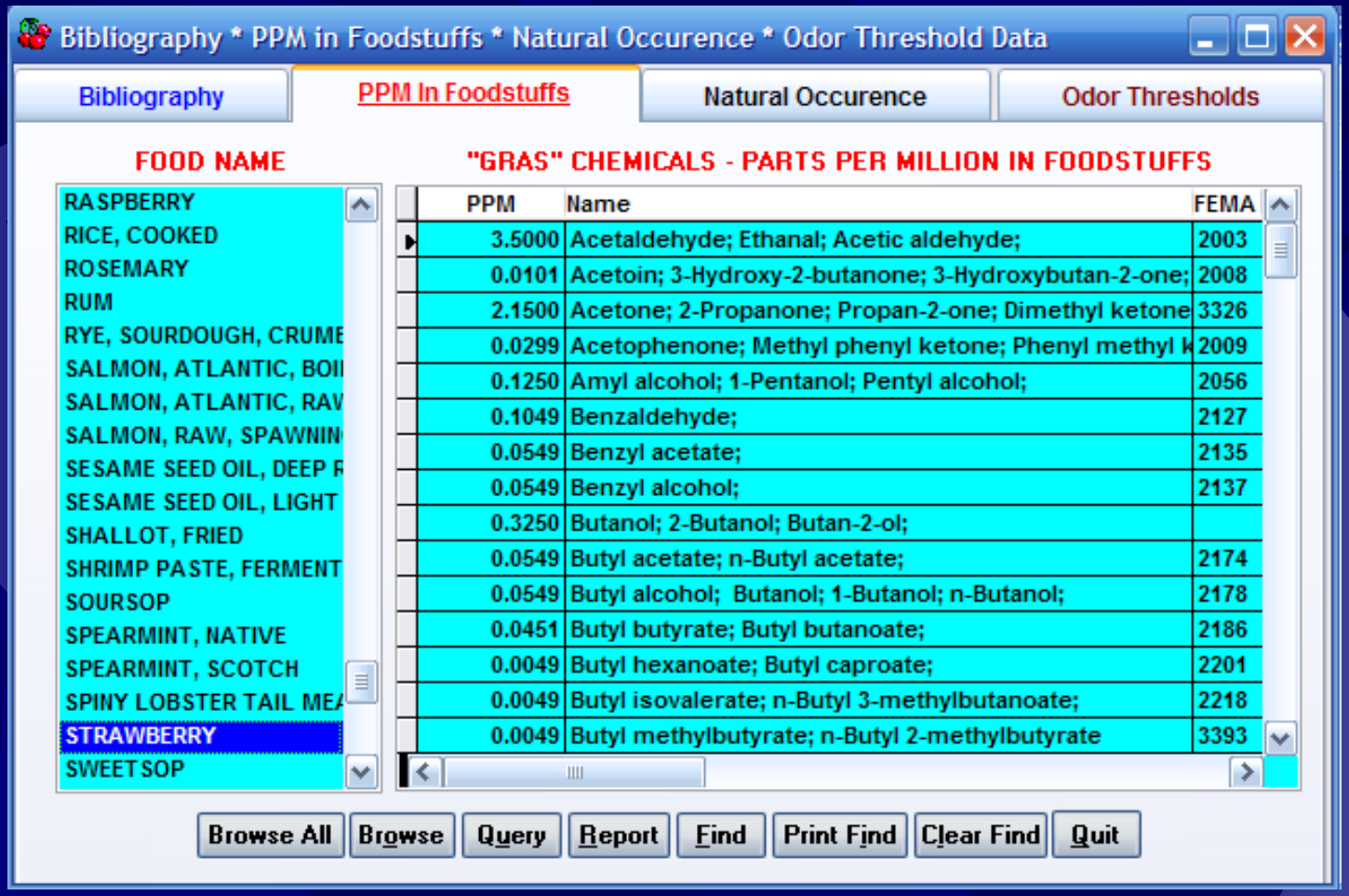

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Report Designer - flavunit.frx - Page 3 - FLAVOR-BASE PRO "2007"

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Report on: STRAWBERRY Page 3

FEMA	COE	FDA	JP	EC REG.	NAME / DESCRIPTION	PPM:	FLAVOR UNIT:
2422	261	172.515	X	09.726	Ethyl benzoate; Threshold in PPB 60.000000 Floral, fruity odor; ylang-ylang like flavor	0.0201	0.33500
2427	264	182.60	X	09.039	Ethyl butyrate; Ethyl butanoate; Threshold in PPB 1.000000 Ethereal, fruity odor; buttery, ripe fruit notes	2.5500	2550.00000
2430	323	172.515	X	09.730	Ethyl cinnamate; Threshold in PPB 17.000000 Sweet, balsamic-cinnamic, honey-like odor and fruity taste	0.0351	2.06470
2432	309	172.515	X	09.059	Ethyl decanoate; Ethyl caprate; Ethyl decylate; Threshold in PPB 44.000000 Sweet, fatty, nut-like, winey-cognac odor	0.0150	0.34090
2439	310	172.515	X	09.060	Ethyl hexanoate; Ethyl caproate; Threshold in PPB 1.000000 Strong, fruity, winey odor; apple, banana, pineapple notes	2.5250	2525.00000
2463	442	172.515	X	09.447	Ethyl isovalerate; Ethyl 3-methylbutanoate; Threshold in PPB 0.400000 Strong, fruity apple odor and taste	0.1549	387.25000
2443	265	172.515	X	09.409	Ethyl methylbutyrate; Ethyl 2-methylbutyrate; Threshold in PPB 0.100000 Strong, green, fruity, apple-strawberry odor and taste	0.5049	5049.00000
2449	392	172.515	X	09.111	Ethyl octanoate; Ethyl caprylate; Ethyl octylate; Threshold in PPB 92.000000 ChemosFruity, winey, sweet odor; cognac-apricot taste	0.1049	1.14021
2456	402	172.515	X	09.121	Ethyl propionate; Ethyl propanoate; Threshold in PPB 9.000000	0.0549	6.10000

Flavunit Record: 42/99 Exclusive

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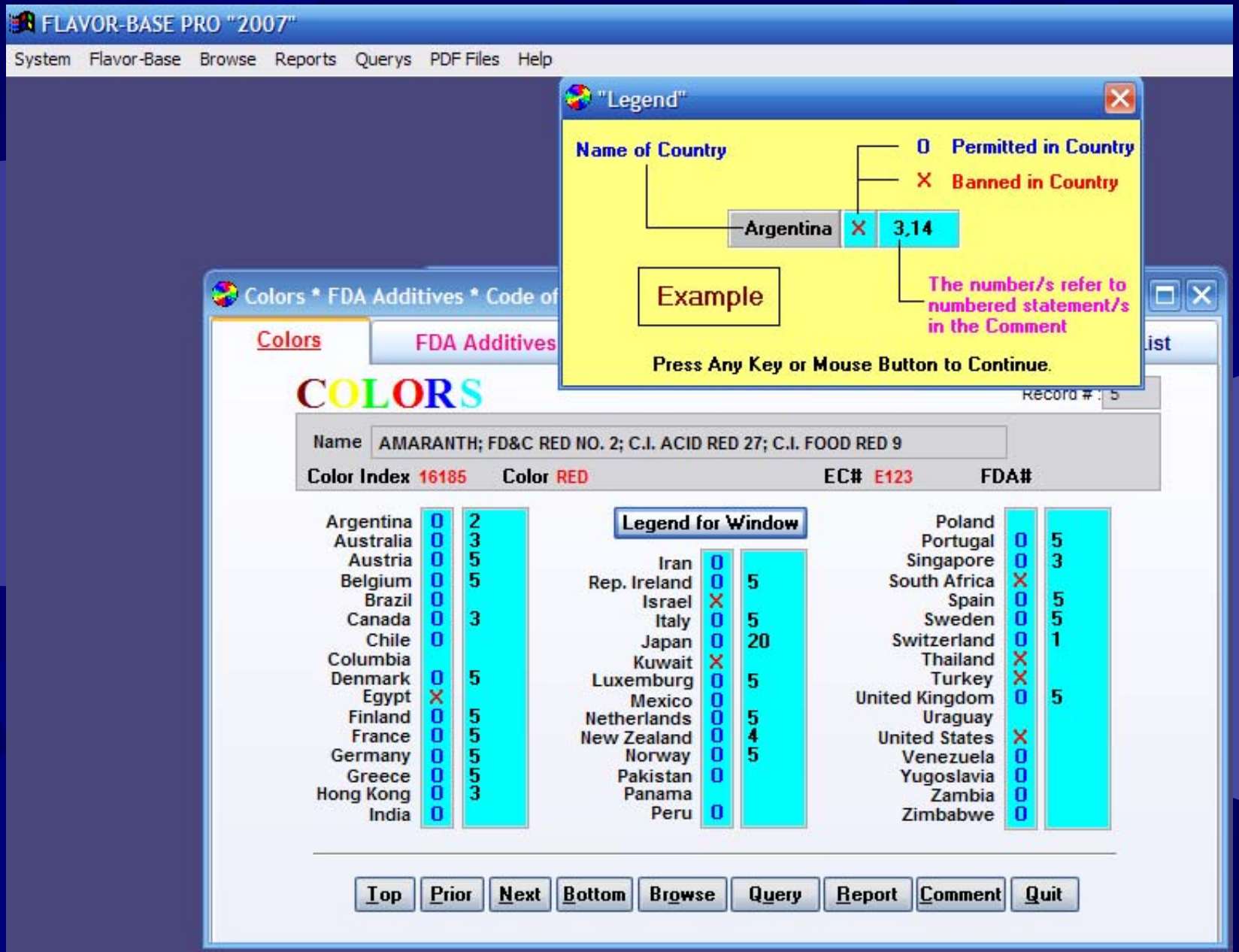

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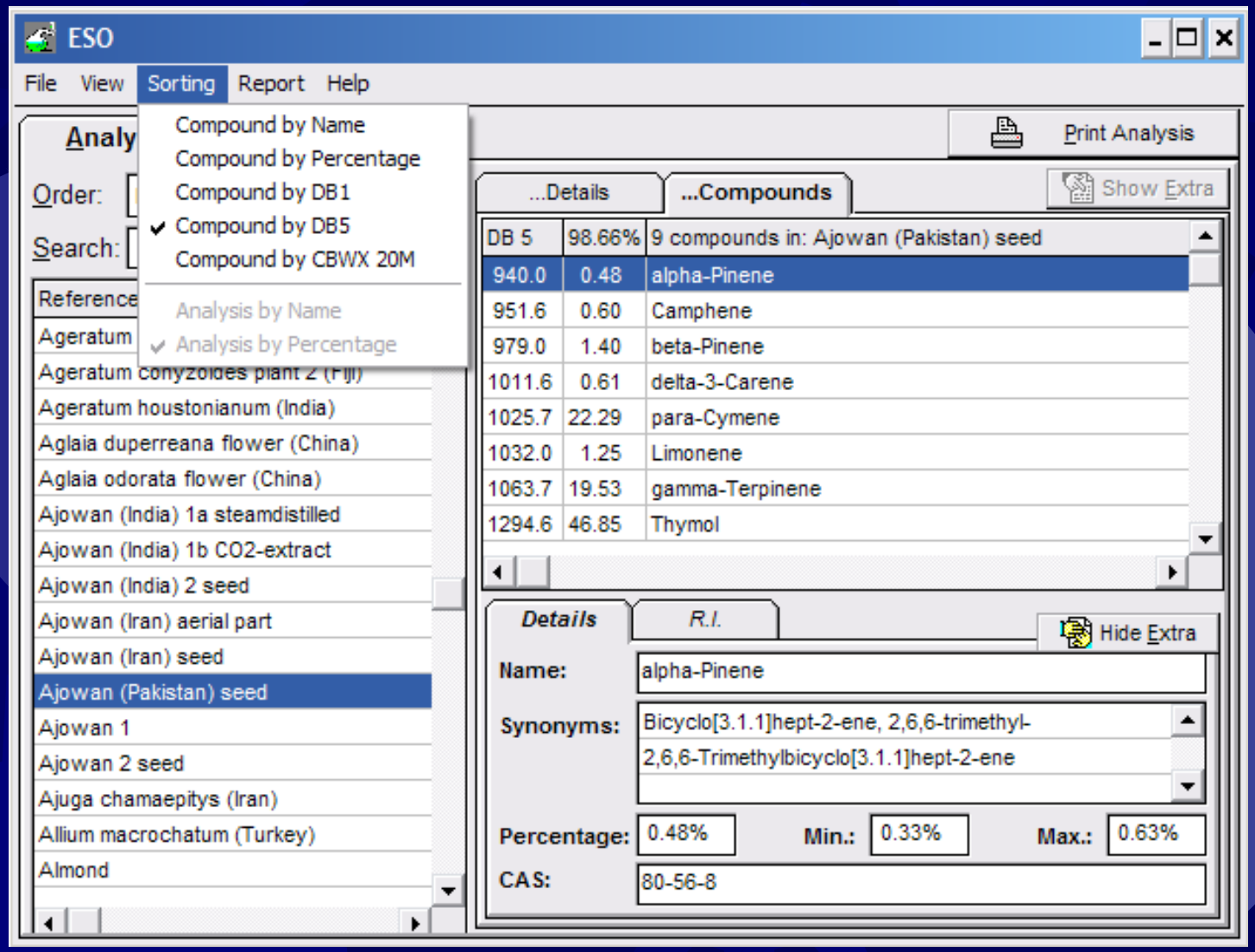
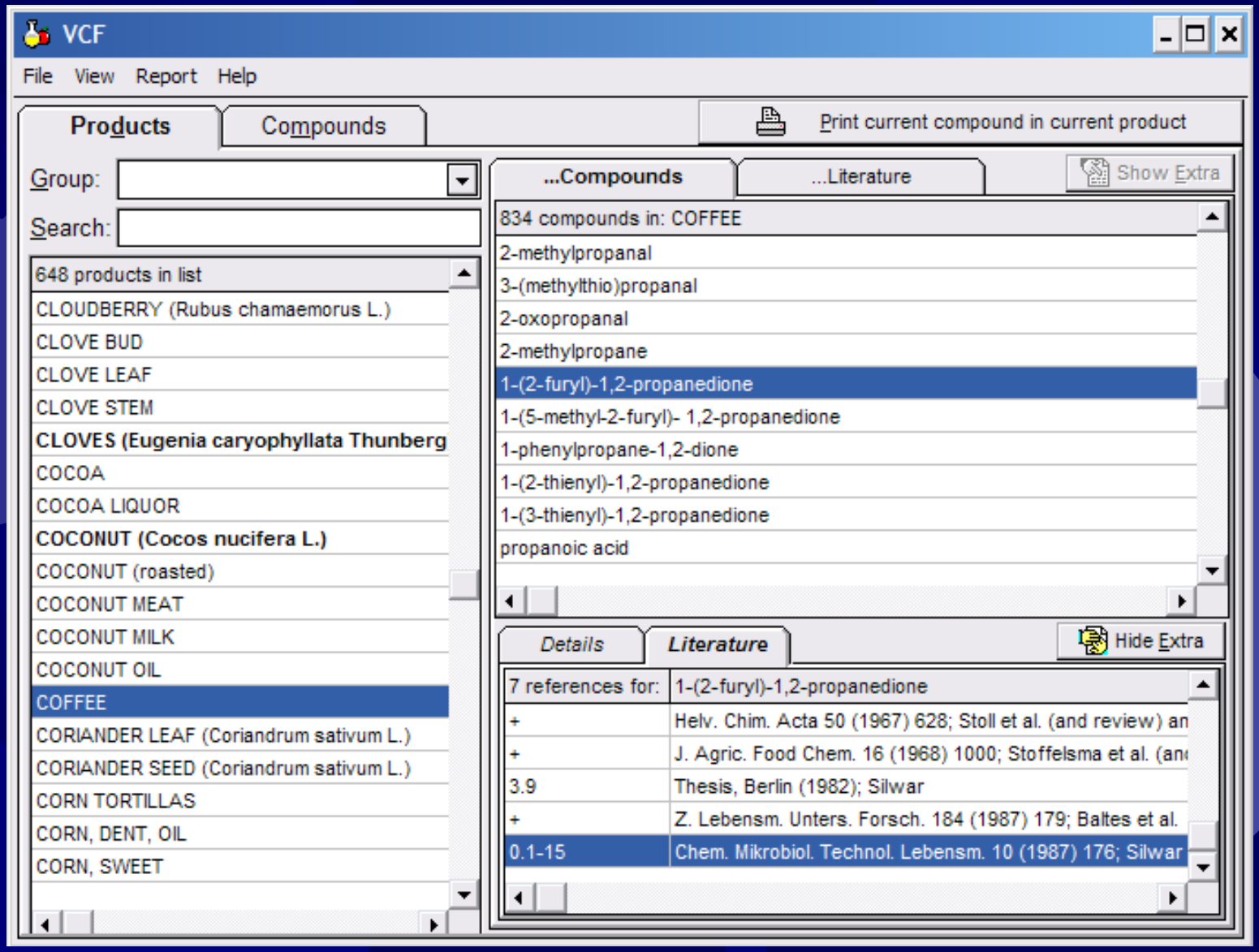
Essential Oils -ESO (update 2006)



Volatile Compounds in Food – VCF 2000



The Final Odds & Trends

GC-MS of *Calvatia gigantea* (The Giant Puffball Mushroom)

The giant puffball (*Calvatia gigantea*) is one of the largest of the edible mushroom species. It is usually found in late summer and autumn in meadows, fields, and forests worldwide. It is only edible when fresh and is usually consumed within 24 hours of harvest as the flavor becomes disagreeable on aging.

GC-MS of *Calvatia gigantea* Headspace

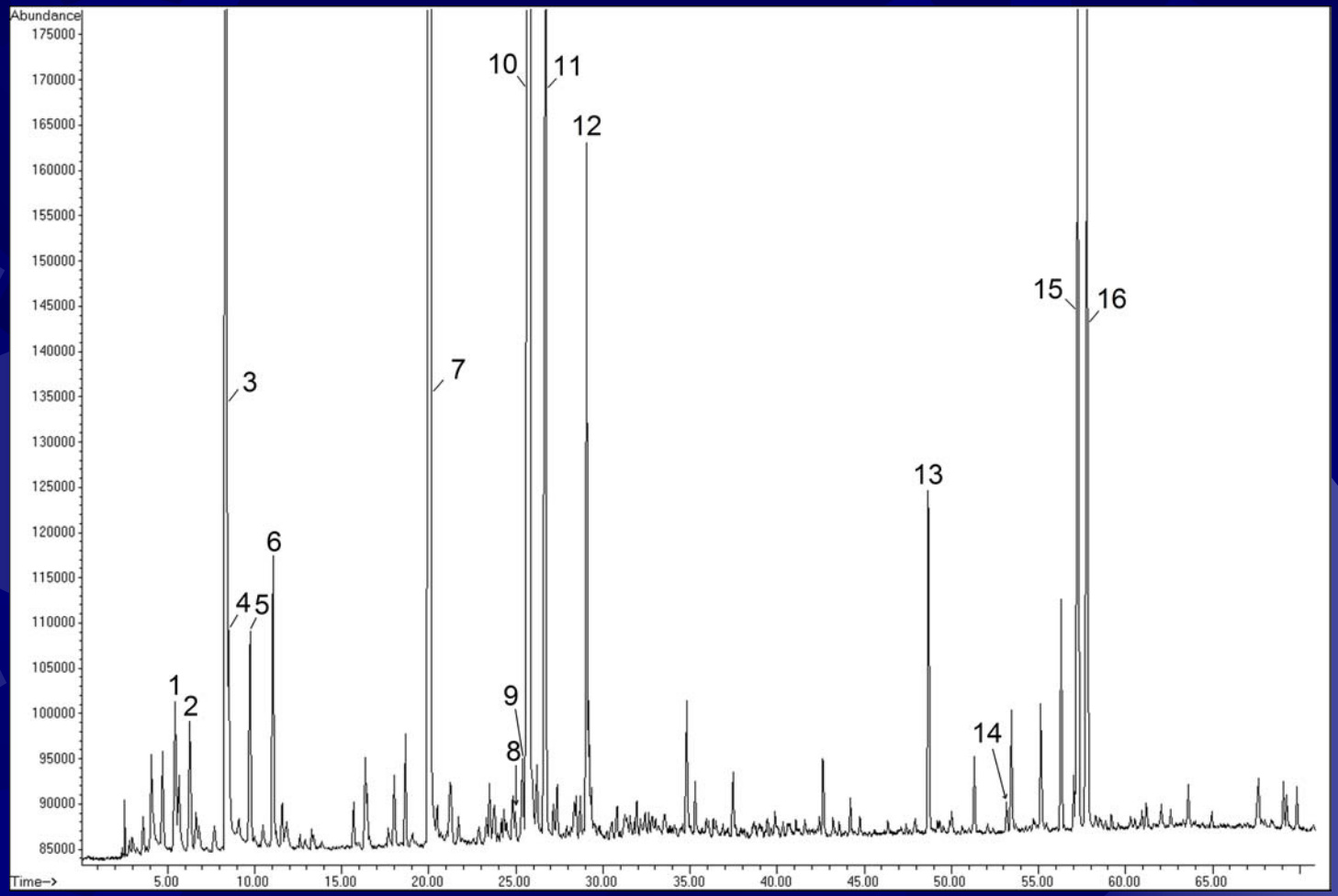
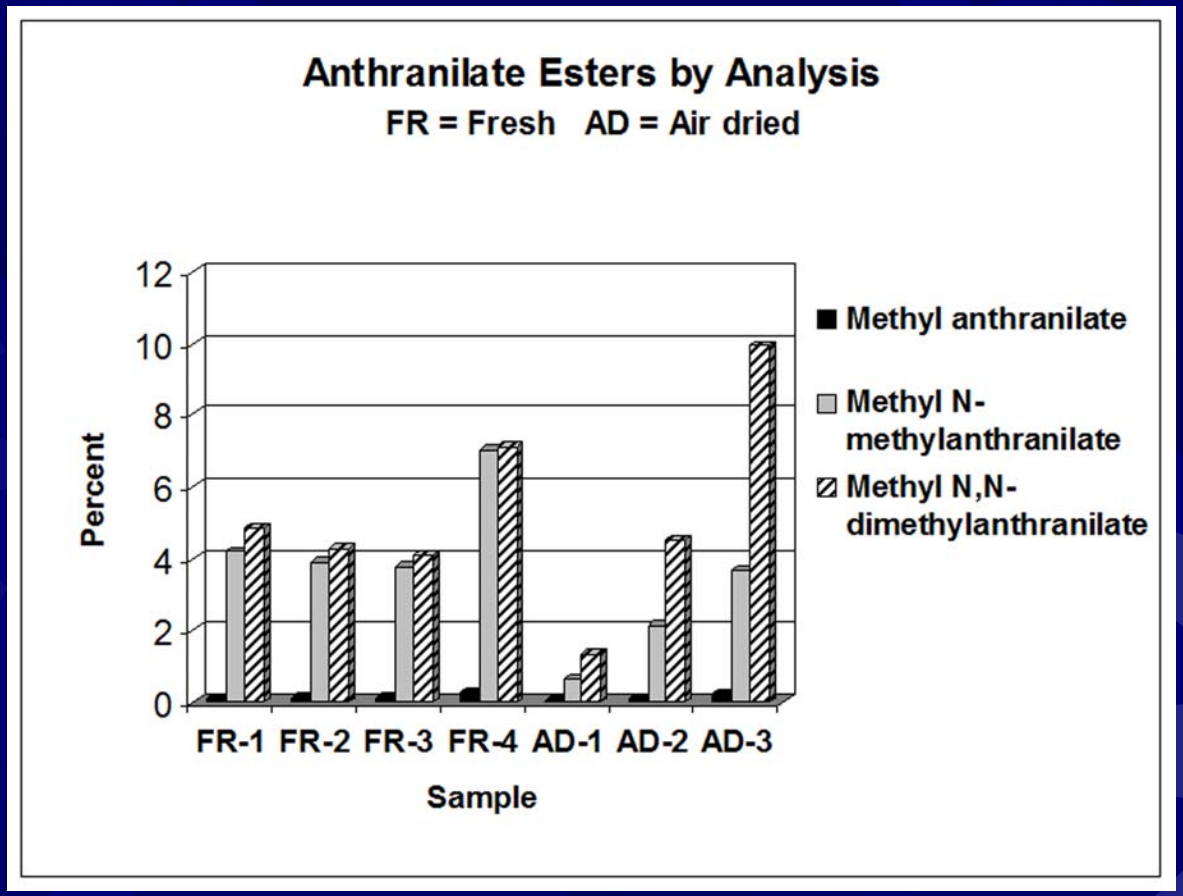


Figure 1. Headspace Chromatogram of Fresh (FR-1) *Calvatia gigantea*
(1) 3-Methylbutanal (2) 2-Pentanone (3) Isoamyl alcohol (4) 2-Methyl-1-butanol (5) Toluene (6) 1-Octene (7) Anisole (8) 1-Octen-3-one (9) 1-Octen-3-ol (10) 3-Octanone (11) 3-Octanol (12) Limonene (13) N-(2-methylphenyl)formamide[tent] (14) Methyl anthranilate (15) Methyl N,N-dimethylanthranilate (16) Methyl N-methylanthranilate

GC-MS of *Calvatia gigantea* Headspace



Significance – First isolation of anthranilates from a mushroom species

Source: Leffingwell & Alford, manuscript in preparation