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GRAS Flavoring Substances 20

The 20th publication by the Expert Panel of the Flavor and Extract Manufacturers Association on recent progress in the consideration of flavoring ingredients generally recognized as safe under the Food Additives Amendment

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In 1958, Congress enacted the Food Additives Amendment (FAA) to the Federal Food, Drug, and Cosmetics Act amid growing concern over the safety of substances added to foods. The FAA set forth standards and guidelines by which the safety of food additives must be established before they can be added to foods (FAA, 1958).

The FAA contained an exclusion provided by Congress for substances "generally recognized, by experts qualified by scientific training and experience to evaluate its safety, as having been adequately shown through scientific procedures . . . to be safe under the conditions of intended use." Based on this, substances "generally recognized as safe" (GRAS) are not considered to be food additives, and are excluded from mandatory premarket approval by the Food and Drug Administration (FDA). However, GRAS substances must meet strict criteria specified by Congress (Degnan, 1991).

As a result, in 1960, the Flavor and Extract Manufacturers Association of the United States (FEMA) created the FEMA GRAS program in which the safety of flavor ingredients would be evaluated for potential GRAS status by an independent panel of experts in the fields of chemistry, toxicology, pharmacology, medicine, pathology, and flavor safety assessment. The conclusions of the Expert Panel would be provided to FDA, the food and flavor industries, and the public. FDA has acknowledged the validity of the FEMA GRAS program and has recognized the FEMA GRAS publications as "reliable industry GRAS lists" within the context of the agency's bulk labeling regulations for flavors codified at 21 CFR Sec. 101.22(b) (2001). FDA expressed significant support for the FEMA GRAS program in the preamble to its proposed voluntary GRAS notification program (FDA, 1997).

The FEMA GRAS lists have been published in *Food Technology* since 1960 (see sidebar on p. 44).

The GRAS assessment performed by the Expert Panel includes a rigorous evaluation of all the available data on flavor ingredients and structurally related substances. The analyses include a comprehensive evaluation of the potential exposure to the flavor ingredients through food compared with toxicologic and pharmacokinetic characteristics. As advancements are made in science, new information becomes available on existing FEMA GRAS flavoring substances. The dynamic FEMA GRAS assessment process incorporates this new information into the program by way of systematic reviews of all GRAS flavor ingredients.

Between 1965 and 1985, the first comprehensive and systematic scientific literature reviews (SLRs) of flavoring substances were completed by FEMA. These SLRs served as the basis for a comprehensive review of substances already designated as FEMA GRAS. This GRAS status reassessment program was known as "GRAS affirmation" or "GRASa" and was completed in 1985.

In 1994, the Expert Panel initiated a second comprehensive reassessment program known as "GRAS reaffirmation" or "GRASr." It is anticipated that this reaffirmation program will be completed in 2005. As part of the GRASr program, the Expert Panel regularly publishes key scientific data on structurally related groups of flavoring substances on which GRAS decisions are based. FEMA GRAS assessments of alicyclic substances, furfural, lactones, and trans-anethole have been published as part of the GRASr program. The fifth in the series, on pyrazine compounds, and the sixth, on methyl eugenol and estragole, have been accepted for publication (Adams et al., 1996, 1997, 1998; Newberne et al., 1999; Smith et al., 2001a, b).

This, the 20th GRAS publication, includes the results of the Expert Panel's review of 60 new GRAS flavoring substances (see pp. 38 and 40, and 45–50). The publication is a landmark in that it contains the 2,000th substance (FEMA No. 4000) to be recognized as GRAS by the FEMA Expert Panel. It also contains the Expert Panel's determination that new use levels and food categories for seven flavoring substances previously considered GRAS are consistent with their current GRAS status (see p. 51). It also includes the panel's views on methods of calculating human dietary exposure to flavoring substances and critically reviews the results of chronic two-year bioassay studies performed at the National Toxicology Program (NTP) for methyl eugenol (FEMA No. 2475) and citral (FEMA No. 2303).

Estimation of Intake/Exposure to Flavoring Substances

As food technology progresses, its impact on the human diet becomes more evident. The global food supply has grown to depend on the quantity, quality, and variety of wholesome and nutritious foods produced through scientific advancements in this field. The use of preservatives, color additives, and flavoring agents by manufacturers plays an important role in sustaining and extending the quality and quantity of food. As a direct result of these advancements, a variety of safety assessment procedures have developed and are currently in place to assure regulators and consumers that food additives and in particular flavoring agents are safe for human consumption (JECFA, 1968, 1996, 1998, 1999, 2000; NAS, 1970, 1980; Oser and Hall, 1977, FSC, 1980; FDA, 1982, 1993; WHO, 1987; SCF, 1991; Hallagan and Hall, 1995; Munro et al., 1999).

The GRAS assessment . . . includes a rigorous evaluation of all the available data on flavor ingredients and structurally related substances.

Exposure or intake of flavoring substances is defined as the amount of substance ingested and is essential to assessing the safety of food ingredients. Quantifying intake of flavoring substances is a daunting task and challenged by many technical and economic difficulties. More than 20,000 different food products are available for consumption in the Western diet (FMI, 1998). These products are occasionally consumed by a large heterogeneous population, which makes it difficult to determine any one individual's intake of a food constituent. Added to this is the difficulty and expense of obtaining accurate intake data, requiring detailed dietary analysis of a large enough group of people to obtain statistically significant results for the diverse population of eaters.

For more than 40 years, government regulators, scientists, and food industry experts have proposed various methods of estimating exposure to flavoring substances in food. Initially, exposure was calculated using a method called "the possible average daily intake" (PADI), which is based on the levels of flavoring substances added to foods and the amounts of those foods consumed (see below). Unfortunately, this approach fails to incorporate the many complexities associated with human consumption patterns and the food supply, usually resulting in exaggerated overestimates of intake.

The current methods for determining exposure to flavoring substances are the estimated PADI in the U.S. and the theoretical added maximum daily intake (TAMDI) in Europe from use of the substance as a flavoring agent. The PADI is determined by (1) multiplying usual use levels of the substance in each of 33 food categories (e.g., baked goods and meat products) by the average amount of that food category consumed daily and (2) summing the intake over all 33 food categories (USDA/ARS, 1973).

For the vast majority of flavoring agents that have low reported annual volumes of use (Lucas et al., 1999; IOFI, 1995), the PADI is a gross exaggeration of the average daily intake. The PADI calculation assumes that all foods in a food category always contain that substance and that the food category is consumed daily (Oser and Hall, 1977). An example of how this assumption can be problematic is ethyl methylphenylglycidate. Since it is added to impart strawberry flavor to hard candy, the PADI method assumes that all hard candy, including peppermints, cherry-flavored lollipops, and butterscotch, contain ethyl methylphenylglycidate.

These methods for calculating intake do not take into account loss of flavoring substance by processing, cooking, or waste. For example, the majority of allyl disulfide, a volatile disulfide, that is added to garlic breads is lost during the baking process. More than 98% of the flavoring substances are low-molecular-weight compounds (<300 Da), so processing (heating) will lead to substantial loss and concomitant lower levels of intake.

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Of the two comprehensive studies of flavor intake undertaken over the past three decades, one involves a detailed dietary analysis (DDA) of a panel of 12,000 consumers, and the other is based on a robust stochastic model (FSM).

The DDA method is based on the detailed reporting of dietary intake of all foods by a panel of consumers over a period of 14 days spread over a year to accommodate seasonal variations in diet (Hall, 1976; Hall and Ford, 1999). Market Research Corp. of America (MRCA) enlisted a diverse panel of 12,000 consumers that came from urban, suburban, and rural communities and ranged in age from infant to over 65 years. The results were statistically analyzed and sorted by age groups and consumption patterns. The participants had more than 4,000 descriptors to choose from for food eaten. Food categories were narrow and highly specific. For example, the baked goods category was divided into 500 subcategories, allowing for garlic bread to not be lumped in with cinnamon coffee cake.

This study assessed the amount of each specific food eaten, the frequency of consumption of each food, the amount of flavoring agent in each food, and the classification of consumer by age, weight, or other pertinent characteristics. The amount of flavoring agent in foods was difficult to determine, since flavor formulas were proprietary. These levels were assured by a panel of food chemists and flavorists familiar with the flavor substance levels in particular food products. Once all the data were compiled, they were taken through eight steps of analysis (Table 1) to produce average intake levels for both eaters-only groups and non-eaters. Eaters are defined as participants who consumed foods containing specific flavoring substances, and non-eaters are consumers with zero reported intake for a particular flavoring substance.

Although this data-intense method is accurate and reliable, it is expensive and time consuming. In the original 1970 survey data gathered on 12 key flavoring substances, two of these substances, cinnamaldehyde ethylene glycol acetal and allyl cyclohexylacetate, exhibited very low intake among the panelists.

Conservative estimates derived from these data are obtained by using the 99th percentile intake levels. In the vast majority of cases, estimates of intake are orders of magnitude lower than those obtained from PADI calculations.

Since DDA methods are economically burdensome, other methodologies to improve the estimation of intake were developed. Based on the results of the DDA study, it was determined that intake could be reliably estimated by applying the *per capita intake × 10* method to the annual production volume of flavoring substances (Rulis et al., 1984; Woods and Doull, 1991). This method assumes that only 10% of the population consumes the total annual reported volume of use of a flavor ingredient. This approximation provides a practical and cost-effective approach to the estimation of intake for flavoring substances. The annual volumes of flavoring agents are relatively easy to obtain by industry-wide surveys, which can be performed on a regular basis to account for changes in food trends and flavor consumption. The 1995 poundage survey of U.S. flavor producers was published by FEMA in 1999 (Lucas et al., 1999).

This method can be evaluated by comparison to the data obtained by the DDA method discussed above. Since the dietary analyses were completed in 1970, it is necessary to use poundage information from that time (NAS, 1972). To correct for possible incompleteness in the poundage survey, these data are assumed to be 60% of the flavoring agents (0.6 correction factor in the equation below) actually used. The per capita daily intake (PCI) in micrograms/day is then calculated from the annual volume, in kilograms, for the U.S. population in 1970 (i.e., 210 million) by the following equation:

$$PCI = \frac{(\text{kg/year})(10^9\ \mu\text{g/kg})}{(210 \times 10^6\ \text{persons})(0.6)(365\ \text{days/year})} = \mu\text{g/person/day}$$

The calculated PCI is then multiplied by 10 to obtain a reasonably conservative estimate for intake by the eaters of the ingredient. The data obtained from PCI × 10 is more conservative than that obtained from the DDA method (see Table 2).

For the 10 substances studied in the panel survey, the PADI is a gross overestimation of the DDA intake. For two high-volume substances, -ionone and methyl salicylate, PADI gave data comparable to PCI × 10. This demonstrates that PADI is a reasonable model to follow for intake estimation of high-volume substances that are used in many food categories. However, for low-volume substances (i.e., allyl disulfide), it gives an estimation three or four orders

Table 1—Steps for determining exposure to food using the DDA method

1. From the Market Research Corp. of America (MRCA) database, obtain the total number of eatings of each specific food (SF), by each panel member, each day over a 14-day period.
2. USDA mean portion size in grams for that person's age group and relevant major food category (= quantity of SF in grams for that person, that day).
3. Weighted mean of the usual use level of the ingredients (I) in the SF in ppm/1,000 (= quantity of I in mg/day for that person, that day from that SF, if all that SF contained I).
4. Probability that the SF actually contains I (= expected intake from the SF for that person, that day in mg).
5. Repeat steps 1–4 for that person for each of the SFs consumed by that person that day (= expected intake of I for each of all SFs consumed by that person, that day in mg)
6. Sum, for each person, intakes of I from all SFs for that day (= expected total intake of I for that person, that day in mg).
7. Intakes in person-days:
   a. To obtain the distribution of the expected daily intakes in person-days for the total panel (eaters and non-eaters), array all of the expected 14 daily intakes of the panelists, calculate the mean, standard deviation, and centiles.
   b. To obtain the distribution of expected daily intakes in person-days for eaters only, disregard all zero person-day intakes and, considering all non-zero daily intakes, calculate the mean, standard deviation, and centiles.
8. 14-day averages:
   a. To obtain the distribution of expected 14-day average intakes for the total panel (eaters and non-eaters), average the daily intakes over the 14-day period for each panelist and, considering all of the 14-day averages, calculate the mean, standard deviation, and centiles.
   b. To obtain the distribution of average intakes for eaters only, disregard all persons with zero average intakes and, considering all non-zero 14-day average intakes, calculate the mean, standard deviation, and centiles.

of magnitude higher than DDA and three orders of magnitude higher than PCI × 10.

A second method that has been developed to improve on our understanding and estimation of exposure to flavoring agents is based on the theoretical full stochastic model, FSM (Cadby, 2001; Lambe, 2000). It was developed to assist the European Union in its goal to evaluate 2,800 flavoring agents by 2004. Some European intake estimates are based on the theoretical added maximum daily intake (TAMDI) paradigm, which is based on the same assumptions as the PADI estimate. It reduces levels of incorporation to 31 different categories of food or beverages. Dietary intake data were collected from British males age 16–24 years in the 1988 Dietary and Nutritional Survey of British Adults. These data provided the maximum concentrations, distributions of concentrations, and the maximum probability of encountering each substance in a flavored food or beverage in any one of the 31 different categories. TAMDI produces intake estimates which are on the same order of magnitude as PADI. If the model is refined and full stochastic treatment of the data is performed (see Table 3), the FSM data are lower than TAMDI estimates by three orders of magnitude for the 12 substances studied (Table 4).

The FSM allows for the complete randomization of conventional intake data and assumes that only a small portion of the population consumes a given flavoring substance at its maximum level on a daily basis. This application of probability to dietary intake provides a more realistic estimation of intake in that it eliminates the exaggeration that the maximum level of added flavoring is consumed daily in each food category. To some extent, even the FSM method overestimates intake in that it does not account for loss due to processing (cooking) or manufacturing waste and the market share of flavored foods in that food category, which has the potential to skew the data depending on the concentration of the substance reported in that food. For example, if a soft drink containing a maximum concentration of isoamyl acetate has a market dominance at the time of data collection, intake for all similar soft drinks will estimate a higher concentration in all cases. If that brand dominance fades, then the familiar scenario of higher intake than manufacture of flavoring agent would occur.

Comparing the FSM and TAMDI methods to the PCI × 10 method (Table 4) reveals that TAMDI, like PADI, overestimates exposure to flavoring agents through food consumption. The PCI × 10 method is a reasonably conservative estimation for safety analysis when compared to the levels of exposure calculated by the FSM. The FSM estimates are comparable to those obtained for 10 different substances by the DDA method with respect to order of magnitude.

The authors of the FSM study pointed out that the PCI × 10 estimates were a close match to the FSM data, which are lower by one order of magnitude in most cases. The probability (pFSM>) of FSM overestimating either TAMDI or PCI × 10 is very small, as shown in Table 4. This analysis affirms that FSM estimates are in good agreement with PCI × 10 estimates.

An advantage to using PCI × 10 estimations is that the common problem shared by other methods of a decreasing supply of flavoring substance being eclipsed and surpassed by intake estimates based on food categories cannot occur. The exposure to flavoring agents is strictly limited to the volume distributed for the use in food. Industry poundage surveys are regularly updated, as are estimations of the population through census.

It can be concluded that the DDA and FSM approaches offer a more realis-

Table 2—Comparison of detailed dietary analysis (DDA), per capita intake × 10 (PCI × 10) and possible average daily intake (PADI) methods for exposure to flavoring agents through food intake

Flavoring Substance	DDA 95th centile intake (µg/day)	Volume (kg/year)	PCI × 10 (µg/day)	PADI (µg/day)
Allyl disulfide	1.4	60	13	2,180
2-Hexenyl acetate	14	60	13	1,480
4-(p-Hydroxyphenyl)butan-2-one	300	3,930	860	2,690
α-Ionone	100	4,430	960	960
Ethyl methylphenylglycidate	1,500	5,090	1,100	22,500
Maltol	3,600	16,600	3,600	29,200
Eugenol	76	22,200	4,800	6,990
Menthol	510	27,500	6,000	13,400
Black pepper oleoresin	5,800	90,900	20,000	289,000
Methyl salicylate	5,400	22,700	49,000	37,100

Table 3—Algorithm used for the full stochastic model for the estimation of exposure to flavoring substances. From Lambe (2000)

Cell	Variable	Input distribution/function
A1	Intake of food A	@RiskHistogram
A2	% of brands of food A containing a flavor	@Risk Discrete (1 or 0)
A3	Chance of encountering flavoring substance in food A	@Risk Discrete (1 or 0)
A4	Presence of flavoring substance in food A	Excel logical function If A2 = 1, A3, 0
A5	Natural log of the concentration of flavoring substance within food A	@RiskHistogram
A6	Exponential of concentration	Excel function Exponential of A5
A6	Actual concentration of flavoring substance in food A	Excel logical function if A4 = 1, A6, 0
A7	Intake of flavoring substance from food A	A1 x A6
	Steps in cells A1–A7 repeated for food B, C, D, etc.	
A100	Total intake of flavoring substance (mg/kg bw/day)	(A7 + A14+ A21+ A28...)/60

tic assessment of intake of flavoring substances through consumption of food. The drawback to the DDA method is the cost and time needed to evaluate the data on a fairly regular basis. The FSM approach requires a computer and a fairly extensive food intake survey as well, which is economically challenging. The PCI × 10 method offers a simple calculation based on easily obtained data, and its results are consistent with those provided by the DDA and FSM methods. Therefore, PCI × 10 offers conservative intake estimates and would be easy to implement on a national and global basis. The Expert Panel uses the PCI × 10 method as a satisfactory means of assessing exposure to flavoring substances.

Safety Assessment of Methyl Eugenol (FEMA 2475)

Methyl eugenol (CAS No. 93-15-2) is 3,4-dimethoxyallylbenzene. It belongs to a group of naturally occurring allylalkoxybenzene derivatives, including estragole and safrole. Methyl eugenol occurs in many foods but is present mainly in spices, including sweet basil, allspice, and nutmeg. It is used as a flavor ingredient in foods up to an average level of 50 ppm. Based on a reported annual volume of 620 kg (Lucas et al., 1999), the estimated per capita intake ("eaters only") is approximately 0.001 mg/kg of body weight/day from use of methyl eugenol as a flavoring substance.

Groups (50 each) of male and female B6C3F1 mice and F344/N rats were administered 0, 37, 75, or 150 mg of methyl eugenol/kg bw in 0.5% methylcellulose by gavage daily, five days per week for two years (NTP, 2000). On completion of the study, NTP concluded:

"Under the conditions of these 2-year gavage studies, there was clear evidence of carcinogenic activity of methyl eugenol in male and female F344/N rats based on increased incidences of liver neoplasms and neuroendocrine tumors of the glandular stomach in male and female rats and increased incidences of kidney neoplasms, malignant mesotheliomas, mammary gland fibroadenoma, and subcutaneous fibroma and fibroma or fibrosarcoma (combined) in male rats. A marginal increase in squamous cell neoplasms of the forestomach may have been related to methyl eugenol administration in female rats. There was clear evidence of carcinogenic activity of methyl eugenol in male and female B6C3F1 mice based on the increased incidences of liver neoplasms in males and females. Neuroendocrine tumors of the glandular stomach in male mice were also considered related to exposure to methyl eugenol.

"In male and female mice and rats, methyl eugenol administration caused significant increases in nonneoplastic lesions of the liver and glandular stomach."

High doses of methyl eugenol and structurally related allylalkoxybenzene derivatives (e.g., estragole and safrole) are carcinogenic in rodents. This has been observed in several different studies in mice and rats, newborns and adults. Repetitive intraperitoneal administration for 20 days of high concentrations of safrole, estragole, or methyl eugenol to preweanling or weanling mice induced liver tumors at approximately 10–12 months (Miller et al., 1982, 1983; Borchert et al., 1973; Wiseman et al., 1987). Preweanling animals were more sensitive to tumorigenesis. Similar effects are seen at higher dose levels with methyl eugenol and safrole administered either by gavage or in the diet (NTP, 2000; Hagan et al., 1967; Long and Jenner, 1963). In these studies, evidence of carcinogenicity was concurrent with evidence of chronic hepatotoxicity. The lowest dose of methyl eugenol administered by gavage at which carcinogenicity and hepatotoxicity were reported in rodents in the NTP two-year bioassay was 37 mg/kg/day. In a separate two-year dietary study, safrole was not carcinogenic when administered in the diet at 25 or 5 mg/kg bw/day, although mild hepatotoxicity was reported even at these dose levels (Long and Jenner, 1963). Therefore, no valid study has as yet been performed in the absence of hepatotoxicity using the oral route of exposure.

Based on the results of these studies, dose-dependent hepatotoxicity induced by methyl eugenol, safrole, and other allylalkoxybenzene derivatives is a necessary step in the formation of hepatic tumors. Daily intakes of methyl eugenol that are carcinogenic in rodents following chronic gavage administration are 1,000-fold higher than the typical dietary intake of methyl eugenol by humans (Lucas et al., 1999; NAS, 1970, 1975, 1981, 1987). Since the amount of methyl eugenol added as a flavoring constituent accounts for approximately 10% of dietary intake, its potential to induce hepatotoxicity is expected to be small, possibly zero. As with all substances administered at high dose in carcinogenic assays, there is uncertainty about the shape of the dose-response curve at low doses that are typical of normal human exposure. This uncertainty is compounded, in the case of

Table 4—Comparison of the use of theoretical added maximum daily intake (TAMDI), PCI × 10, and the full stochastic model (FSM) to estimate the intake of selected flavoring substances (μg/kg bw/day) and the probability that intakes along the distribution of FSM would exceed the TAMDI or PCI × 10 estimates (pFSM>).

Flavoring substance	TAMDI	PCI × 10	FSM (97.5th %ile)	pFSM> TAMDI	pFSM> PCI × 10
Isoamyl acetate	1,993	380	36.6	<0.0001	0.0011
Carvyl acetate	193	0.08	0	<0.0001	0.0044
delta-Decalactone	97	140	11.7	0.0011	0.0004
Dihydrocarveol	1,714	0.007	0	<0.0001	0.0019
Furfuryl alcohol	97	3.42	0.35	<0.0001	0.0050
Isopulegol	161	0.12	0.006	<0.0001	0.0070
Acetyl methyl carbinol	225	45.9	15.9	0.0002	0.0054
Allyl caproate	35.5	43.3	3.4	0.0037	0.0029
Eucalyptol	42.1	24	0.16	0.0007	0.0010
2,6-Dimethyl pyrazine	103	0.026	0.24	<0.0001	0.0753
Methyl thiobutyrate	16	0.057	0.048	<0.0001	0.0221
2-Acetyl pyridine	32.1	0.98	0.22	0.0001	0.0083

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methyl eugenol, by the fact that it was administered chronically in the NTP bioassay by gavage, which clearly induced gastric toxicity.

Methyl eugenol has been detected in the blood plasma of humans (Barr et al., 2000), indicating that it and other structurally related substances are absorbed from the diet and distributed. It is rapidly metabolized by O-demethylation (Sangster et al., 1983, 1987), epoxidation (Delaforge et al., 1980), and 1'-hydroxylation, with the 1'-hydroxymetabolite being the proximate hepatotoxic and carcinogenic agent (Drinkwater et al.,

Table 5—Primary names (in boldfaced capital letters, listed alphabetically) and synonyms (in lower case)

FEMA No.	Substance primary name and synonyms
3964	**2-ACETYL-3-METHYLPYRAZINE** Ethanone, 1-(3-methylpyrazinyl)- 1-(3-Methylpyrazinyl) ethan-1-one 2-Methyl-3-acetylpyrazine 3-Acetyl-2-methylpyrazine Ketone, methyl 3-methylpyrazinyl Methyl acetyl pyrazine-2,3
3965	**1-AMINO-2-PROPANOL** Isopropanolamine (RS)-1-Amino-2-propanol DL-1-Amino-2-propanol *alpha*-Aminoisopropyl alcohol *beta*-Aminoisopropanol 1-Amino-2-hydroxypropane 1-Methyl-2-aminoethanol 2-Hydroxy-1-methylethanol 2-Hydroxy-1-propylamine threamine
3966	**3-DECANONE** Decan-3-one Ethyl heptyl ketone
3967	***CIS*-4-DECENYL ACETATE** 4-Decen-1-ol, acetate, (Z)-
3968	**DIISOPROPYL TRISULFIDE** Bis(1-methylethyl)trisulfide 2,6-Dimethyl-3,4,5-trithiaheptane
3969	**(E) & (Z)-4,8-DIMETHYL-3,7-NONADIEN-2-ONE** Citronone
3970	**2,5-DIMETHYL-3-OXO-(2H)-FUR-4-YL BUTYRATE** Butanoic acid, 4,5-dihydro-2,5-dimethyl-4-oxo-3-furanyl ester 4-Butyroxy-2,5-dimethyl-3(2H)-furanone
3971	***CIS AND TRANS*-2,5-DIMETHYLTETRAHYDROFURAN-3-THIOL** 3-Furanthiol, tetrahydro-2,5-dimethyl-
3972	***CIS AND TRANS*-2,5-DIMETHYLTETRAHYDRO-3-FURYL THIOACETATE** Ethanethioic acid, S-(tetrahydro-2,5-dimethylfuranyl)ester
3973	**ETHANETHIOIC ACID, S-(2-METHYL-3-FURANYL) ESTER** 3-(Acetylthio)-2-methylfuran
3974	**ETHYL 4-(ACETYLTHIO)BUTYRATE** Butanoic acid, 4-(acetylthio)-, ethyl ester
3975	**ETHYL *CIS*-4-HEPTENOATE** 4-Heptenoic acid, ethyl ester (Z)-Ethyl *cis*-hept-4-enoate *cis*-4-Heptenoic acid ethyl ester
3976	**ETHYL 5-HEXENOATE** 5-Hexenoic acid, ethyl ester Ethyl hex-5-enoate
3977	**(+/-) ETHYL 3-MERCAPTOBUTYRATE** 3-Mercaptobutyric acid, ethyl ester
3978	**ETHYL 5-(METHYLTHIO)VALERATE** Pentanoic acid, 5-(methylthio)-,ethyl ester
3979	**FURFURYL PROPYL DISULFIDE** Furan, 2-[(propyldithio)methyl]-
3980	**(+/-) HEPTAN-3-YL ACETATE** 3-Heptanol, acetate Hept-3-yl acetate 1-Ethylpent-1-yl acetate Acetic acid, 3-heptyl ester
3981	**(+/-) HEPTAN-2-YL BUTYRATE** Butanoic acid, 1-methylhexyl ester Hept-2-yl butyrate Butanoic acid, 2-heptyl ester
3982	**(Z)-3-HEXENYL (E)-2-BUTENOATE** 2-Butenoic acid, 3-hexenyl ester (E,Z)-Crotonate de (Z)-3-hexenyle (Z)-3-Hexenyl crotonate (Z)-3-Hexenylcrotonat (E,Z)-2-Butenoic acid 3-hexenyl ester *cis*-3-Hexenyl *trans*-2-butenoate
3983	**(E)-2-HEXENYL HEXANOATE** Hexanoic acid, (2E)-2-hexenyl ester *trans*-2-Hexenyl caproate *trans*-2-Hexenyl hexanoate
3984	**4-HYDROXYBENZALDEHYDE** 4-Formylphenol p-Formylphenol p-Oxybenzaldehyde
3985	**2-HYDROXYBENZOIC ACID** Salicylic acid 2-Carboxy phenol 2-Hydroxybenzene carboxylic acid
3986	**4-HYDROXYBENZOIC ACID** p-Hydroxybenzoic acid 4-Carboxyphenol
3987	**4-HYDROXYBENZYL ALCOHOL** (4-Hydroxyphenyl) methanol p-(Hydroxymethyl) phenol p-Hydroxybenzyl alcohol 4-Hydroxybenzene methanol
3988	**4-HYDROXY-3-METHOXYBENZOIC ACID** Vanillic acid m-Anisic acid, 4-hydroxy
3989	**3(2)-HYDROXY-5-METHYL-2(3)-HEXANONE** 2(3)-Hexanone, 3(2)-hydroxy-5-methyl
3990	**ISOPENTYLIDENE ISOPENTYLAMINE** N-(3-Methylbutylidene)-3-methyl-1-butylamine N-Isoamylidene-isoamylamine 1-Butanamine, 3-methyl-N-(3-methylbutylidene)-
3991	**ISOPRENYL ACETATE** 3-Methyl-3-butenyl acetate
3992	**D,L-MENTHOL(+/-)-PROPYLENE GLYCOL CARBONATE** Frescolat, Type MPC (racemic) Carbonic acid, 2-hydroxypropyl-5-methyl-2-(1-methylethyl)cyclohexylester 5-Methyl-2-(1-methylethyl)-2-hydroxy propyl carbonic acid cyclohexyl ester
3993	***ERYTHRO AND THREO*-3-MERCAPTO-2-METHYLBUTAN-1-OL** 1-Butanol, 3-mercapto-2-methyl 3-Mercapto-2-methylbutyl alcohol
3994	**3-MERCAPTO-2-METHYLPENTANAL** Pentanal, 2-methyl-3-mercapto
3995	**(+/-)2-MERCAPTO-2-METHYLPENTAN-1-OL** 1-Pentanol, 2-mercapto-2-methyl

Table 5 continued on page 44 ▶

1976; Solheim and Scheline, 1973; Zangouras et al., 1981). The daily production of 1'-hydroxymetabolite by rodents at high dose levels in chronic studies is orders of magnitude greater than those formed in humans at typical dietary intake. Less than 0.3% of a typical dietary dose of estragole is metabolized and excreted in the urine of humans as the 1'-hydroxymetabolite, while as much as 40% of carcinogenic doses of safrole can be accounted for in the urine of mice as the 1'-hydroxymetabolite. The increase in 1'-hydroxylation has been related to dose-dependent induc-

Table 5—Primary names and synonyms, *continued*

FEMA No.	Substance primary name and synonyms
3996	**3-MERCAPTO-2-METHYLPENTAN-1-OL (RACEMIC)** 1-Pentanol, 2-methyl-3-mercapto
3997	**4-MERCAPTO-4-METHYL-2-PENTANONE** Thiomethyl pentanone-4,4,2 2-Mercapto-2-methylpentan-4-one 4-Methyl-4-mercapto-2-pentanone
3998	**(+/-) 2-METHYL-1-BUTANOL** 2-Methyl-n-butanol 2-Methylbutyl alcohol Active amyl alcohol Active primary amyl alcohol Primary active amyl alcohol sec-Butylcarbinol
3999	**(+/-) 3-METHYL-*GAMMA*-DECALACTONE** 2(3H)-Furanone, 5-hexyldihydro-4-methyl-(9CI) 5-Hexyldihydro-4-methylfuran-2(3H)-one
4000	**2-METHYLHEPTAN-3-ONE** 3-Heptanone, 2-methyl 2-Methyl-3-heptanone Butyl isopropyl ketone
4001	**(E)-6-METHYL-3-HEPTEN-2-ONE** 3-Hepten-2-one, 6-methyl- *trans*-6-Methylhept-3-en-2-one
4002	**METHYL 2-METHYL-2-PROPENOATE** 2-Propenoic acid, 2-methyl-, methyl ester Methyl 2-methacrylate, 2-(methoxycarbonyl)-1-propene
4003	**METHYL (METHYLTHIO)ACETATE** Acetic acid, (methylthio)-, methyl ester Methyl 2-(methylthio)acetate (Methylthio)acetic acid methyl ester
4004	**2-(METHYLTHIO)ETHANOL** *beta*-(Methylthio)ethanol *beta*-Hydroxyethyl methyl sulfide *beta*-Methylmercaptoethanol 2-Hydroxyethyl methyl sulfide 2-Methylmercaptoethanol Hydroxyethyl methyl sulfide Methyl 2-hydroxyethyl sulfide S-Methylmercaptoethanol
4005	**12-METHYLTRIDECANAL**
4006	**L-MONOMENTHYL GLUTARATE** Pentanedioic acid, mono(5 menthyl-2-1(1-methylethyl)cyclohexyl]ester[1L] [1R(-)] Monomenthyl glutarate
4007	**(+/-) NONAN-3-YL ACETATE** 3-Nonanol, acetate Non-3-yl acetate 1-Ethylhept-1-yl acetate
4008	**(E,E)-3,5-OCTADIEN-2-ONE** Octa-3,5-dien-2-one *trans*, *trans*-3,5-Octadien-2-one
4009	**(+/-) OCTAN-3-YL FORMATE** 3-Octanol, formate Oct-3-yl formate 1-Ethylhex-1-yl formate
4010	**PARALDEHYDE** s-Trioxane 2,4,6-Trimethyl-1,3,5-trioxane Acetaldehyde, trimer Elaldehyde Paracetaldehyde Paral 2,4,6-Trimethyl-1,3,5-trioxacyclohexane
4011	**4-PENTENYL ACETATE** 4-Penten-1-ol, acetate 4-Penten-1-yl acetate 5-Acetoxy-1-pentene 1-Acetoxy-4-pentene
4012	**2-PENTYL ACETATE** 2-Pentanol acetate
4013	**PERILLA LEAF OIL** Shiso Oil
4014	**PHENETHYL ISOTHIOCYANATE** Benzene, (2-isothiocyanatoethyl)- Isothiocyanic acid, phenethyl ester *beta*-Phenethyl isothiocyanate *beta*-Phenylethyl isothiocyanate 2-Phenylethyl isothiocyanate Phenethyl mustard oil Phenylethyl mustard oil
4015	**PYRAZINE** p-Diazine 1,4-Diazine Piazine Paradiazine 1,4-Diazabenzene
4016	**SODIUM 4-METHOXYBENZOYLOXYACETATE**
4017	**2,4,6-TRIISOBUTYL-5,6-DIHYDRO-4H-1,3,5-DITHIAZINE** 4H-1,3,5-Dithiazine, dihydro-2,4,6-tris(2-methylpropyl)-
4018	**2,4,6-TRIMETHYLDIHYDRO-4H-1,3,5-DITHIAZINE** Thialdine 4H-1,3,5-Dithiazine, dihydro-2,4,6-trimethyl-(2α, 4α, 6α)- 2,4,6-Trimethyldihydro-1,3,5-dithiazine 2,4,6-Trimethylperhydro-1,3-dithiazine 2,6-Dihydro-2,4,6-trimethyl-1,3,5-dithiazine Dihydro-2,4,6-trimethyl-1,3,5(4H)dithiazine
4019	**3,7,11-TRIMETHYL-2,6,10-DODECATRIENAL** 3,7,11-Trimethyl dodecatrien-2,6,10-al-1 Farnesal
4020	**(+/-)-(2,6,6-TRIMETHYL-2-HYDROXYCYCLOHEXYLIDENE)ACETIC ACID GAMMA-LACTONE** (+/-) Dihydroactinidiolide 5,6,7,7α-Tetrahydro-4,4,7α-trimethyl-2(4H)benzofuranone
4021	**2,3,5-TRITHIAHEXANE** Trithiahexane, 2,3,5- Methyl (methylthio) methyl disulfide (Methyldithio) (methylthio) methane 2,4,5-Trithiahexane
4022	**6-UNDECANONE** Undecan-6-one Diamyl ketone Dipentyl ketone
4023	**VANILLIN *ERYTHRO AND THREO*-BUTAN-2,3-DIOL ACETAL** Phenol, 4-(4,5-dimethyl-1,3-dioxolan-2-yl)-2-methoxy-

tion of selected CYP-450 isoenzymes (2E1) (Sharma et al., 2001).

Following metabolism, methyl eugenol forms adducts to DNA (Miller et al., 1983; Phillips et al., 1984; Randerath et al., 1984; Wiseman et al., 1987) and protein (Gardner et al., 1997; Sangster et al., 1983, 1987; Zangouras et al., 1981). Dose-dependent protein adducts have been isolated from rats receiving repeated doses of methyl eugenol (Gardner et al., 1997). These have not been chemically characterized. The principal DNA adduct originates from the 1'-hydroxymetabolite through coupling of an allylic carbocation to the exocyclic amino group (N^2) of deoxyguanosine residues. DNA adducts have been detected in the livers of mice at doses that induce tumor formation. Comparison of adduct levels induced in preweanling and weanling mice and the kinetics of their disappearance indicate that these adducts are more slowly removed from preweanling animals. This may explain the increased sensitivity of newborns to the carcinogenic effects of high dose levels of methyl eugenol.

Curiously, methyl eugenol is not strongly mutagenic in bacterial or yeast test systems with metabolic activation (Dorange et al., 1977; Mortelmans et al., 1986; Schiestl et al., 1989; Sekizawa and Shibamoto, 1982; To et al., 1982). 1'-Acetoxymethyleugenol, a chemical model for the ultimate activation metabolite, 1'-sulfooxymethyleugenol, is mutagenic in *Salmonella*, although a nonlinear dose-response was reported (Boberg et al., 1983; Drinkwater et al., 1976; Gardner et al., 1995, 1996; Miller et al., 1982, 1983; Swanson et al., 1981). The mutagenic potencies of the N^2-deoxyguanosine adducts of methyl eugenol have not been directly tested in site-specific mutagenesis assays. Structurally analogous DNA adducts formed by reaction of the epoxide of sytrene oxide with the exocyclic amino group of deoxyadenosine are weakly mutagenic in site-specific assays (Latham et al., 1993). Their activities in comparable assays are approximately 10- to 100-fold lower than that of strongly mutagenic DNA adducts such as O^6-methyldeoxyguanosine. It would be highly desirable to quantify, in parallel, the levels of methyleugenol-DNA adducts in the liver as a surrogate for genotoxicity.

In conclusion, the qualitative and quantitative aspects of the molecular disposition of methyl eugenol and estragole and their associated toxicological sequelae have been relatively well defined from mammalian studies. Several studies have clearly established that the profiles of metabolism, metabolic activation, and covalent binding are dose dependent and that their relative importance diminishes markedly at low levels of exposure (i.e., these events are not linear with respect to dose). In particular, rodent studies show that these events are minimal, probably in the dose range of 1–10 mg/kg bw, which is approximately 100–1,000 times the anticipated human exposure to these substances. For these reasons, it is conclud-

ed that present exposure to methyl eugenol and estragole resulting from consumption of food, mainly spices and added as such, does not pose a significant cancer risk. Nevertheless, in the interim, further studies are needed to confirm both the nature and implications of the dose-response curve in rats at low levels of exposure to methyl eugenol and estragole.

Safety Assessment of Citral (FEMA No. 2045)

Citral is an aliphatic terpene aldehyde that occurs naturally in lemons, oranges, tomatoes, and many other fruits. Chemically, it is a mixture of the cis and trans isomers of 3,7-dimethyl-2,6-octadienal (CAS No. 5392-40-5). It is used as a flavor ingredient in foods up to an average level of 200 ppm. Based on a reported annual volume of 53,200 kg (Lucas et al., 1999), the estimated per capita intake ("eaters only") of citral is 0.092 mg/kg bw/day.

A bioassay on citral was conducted by the Battelle Columbus Laboratory under contract to the National Toxicology Program (NTP, 2001). Groups of 50 F344 rats of both sexes were administered diets containing 0, 1,000, 2,000, or 4,000 ppm of microencapsulated citral for two years. These dietary levels were estimated to provide an average daily intake of 0, 50, 100, or 210 mg/kg. Groups of B6C3F1 mice were administered diets containing 0, 500, 1,000, or 2,000 ppm of citral, estimated to provide average daily intake levels of 0, 60, 120, or 260 mg/kg, for two years. On May 3, 2001, the NTP Board of Scientific Counselors Technical Report Review Subcommittee met for a peer review of the recently issued draft "NTP Technical Report on Citral" (NTP, 2001). The subcommittee concluded:

"Under the conditions of these 2-year feed studies there was no evidence of carcinogenic activity of citral in male or female rats exposed to 1,000, 2,000, or 4,000 ppm. There was no evidence of carcinogenic activity of citral in male B6C3F1 mice exposed to 500, 1,000, or 2,000 ppm. There was equivocal evidence of carcinogenic activity in female B6C3F1 mice based on increased incidences of malignant lymphoma."

The neoplastic response reported in the NTP study was a dose-related increase in the incidence of lymphoma that was statistically significant in the high dose in B6C3F1 female mice—P = 0.011 by Fisher exact test; 12/50 (24%) at 2,000 ppm vs 3/50 (6%) in controls. There was no evidence of increased incidence of malignant lymphoma in either sex of F344/N rats, in male B6C3F1 mice, or in the low- and mid-dose levels in female B6C3F1 mice.

The background incidence of malignant lymphoma in control female B6C3F1 mice maintained on an NTP-2000 diet is high (98/659), with a historical incidence of 14.0% (standard deviation, ±7.1%) and a range of 6–30% (NTP, 2001). The incidence of spontaneous malignant lymphoma in female B6C3F1 mice in all two-year rodent carcinogenicity studies carried out by NTP is also high (20.9%) (Haseman et al., 1998). The historical incidence in controls maintained on the NIH-07 diet at the same contract laboratory performing the citral study was high (167/953), with a historical incidence of 17.5% (standard deviation, 7.7%) and a range of 6–30%.

Therefore, these tumors occur at a high and variable rate in control animals. It is recommended (Haseman et al., 1986) that a compound is anticipated to exhibit a carcinogenic potential if the highest dose is associated with an increased incidence of a common tumor that is significant at the 1% ($P < 0.01$) level, or an increased incidence in a rare tumor at the 5% ($P < 0.05$) level. Therefore, statistical analysis should apply a significance level of 1% ($P < 0.01$) to account for the high background incidence of lymphomas in female B6C3F1 mice. Based on pair-wise comparisons of the incidence of malignant lymphoma in the NTP study by a Fisher exact test, the incidence of this commonly observed neoplasm is not considered to be statistically significant ($P = 0.011$) for female mice at the 1% level.

Decreased body weights in female mice exposed to 500 (after week 30), 1,000, or 2,000 ppm of citral in the diet also confounded the interpretation of the neoplastic response in female mice. The lack of any significant decrease in feed consumption in these groups suggests that the dose-dependent decrease in body weights is evidence of toxicity.

Based on the high frequency of this neoplastic response in historical controls in NTP studies (Haseman et al., 1998), the fact that toxicity was observed at all dose levels in female B6C3F1 mice, and the observation that the incidences of lymphoma reported in the NTP study were not significant at the 1% level ($P < 0.01$) (Haseman et al., 1986), the FEMA Expert Panel concludes that the results of the NTP bioassay do not provide evidence that citral is a carcinogenic risk to humans. The lack of any evidence of carcinogenicity in both sexes of F344 rats and male B6C3F1 mice support this conclusion.

The FEMA Expert Panel concludes that citral is generally recognized as safe (GRAS) under conditions of intended use as a flavoring substance and that use does not present a carcinogenic hazard to humans.

Expert Panel Member Changes

In January 2000, Lawrence J. Marnett, Professor of Biochemistry at Vanderbilt University School of Medicine, joined the panel. In December 1999, Paul M. Newberne, Professor Emeritus in the Dept. of Pathology at Boston University School of Medicine and former Co-Chair of the Expert Panel, retired from the panel after a distinguished tenure. John Doull, Professor Emeritus, University of Kansas Medical School, retired from the panel in December 1999 but continues on as a consultant to the panel in key areas of expertise. Ian C. Munro, Consultant, Toxicologist and Principal, Cantox Health Sciences, Inc., retired from the panel in May 2000 but also continues as a consultant to the panel in areas of key expertise.

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Tables 6 and 7 on pp. 49–55

Table 6—Use levels for new FEMA GRAS flavoring substances on which the FEMA Expert Panel based its judgments that the substances are generally recognized as safe (GRAS)

	Average usual ppm/Average maximum ppm									
	1	2	3	4	5	6	7	8	9	10
	2-Acetyl-3-methyl-pyrazine	1-Amino-2-propanol	3-Decanone	*cis*-4-Decenyl acetate	Diisopropyl trisulfide	(E) & (Z)-4,8-Dimethyl-3,7-nonadien-2-one	2,5-Dimethyl-3-oxo-(2H)-fur-4-yl butyrate	*cis and trans*-2,5-Dimethyltetra hydrofuran-3-thiol	*cis and trans*-2,5-Dimethyltetra hydro-3-thioacetate	Ethanethioic acid, S-(2-methyl-3-furanyl) ester
Category	**FEMA No. 3964**	**3965**	**3966**	**3967**	**3968**	**3969**	**3970**	**3971**	**3972**	**3973**
Baked goods	1.3/4.3	0.01/0.1	8/15	4/8	5/15	50/300	8/16	0.4/0.8	3/6	5/10
Beverages (nonalcoholic)	0.3/0.6	0.03/0.1	5/10	2/4	0.5/4	1/10	4/8	0.2/0.4	1/2	0.1/1
Beverages (alcoholic)	0.3/3	0.03/0.1	5/10	2/4	1/8	5/40	4/8	0.2/0.4	1/2	
Breakfast cereal	0.1/2					1/10			3/6	0.1/0.5
Cheese										0.01/0.1
Chewing gum	0.8/8		30/60	10/20	5/15	50/250	20/40	1/2	4/8	
Condiments/ relishes								0.2/0.4	1/2	0.001/0.01
Confectionery frostings	0.3/3	0.02/0.2			1/5	5/40				
Egg products	0.3/3				1/5	5/40				
Fats/oils	0.3/3				1/5	30/150				0.5/5
Fish products	0.1/3					5/40				0.5/5
Frozen dairy	1/5	0.02/0.2	6/12	3/6	1.4/6	10/60	6/12	0.3/0.6	2/4	
Fruit ices	0.2/2				0.8/4	10/60	4/8	0.2/0.4	2/4	
Gelatins/ puddings	0.2/2	0.02/0.2			0.8/4	10/60	6/12		2/4	
Granulated sugar										
Gravies	0.3/3			2/4	1/6	5/30	4/8	0.1/0.2	2/4	0.5/5
Hard candy	0.5/5	0.02/0.2	8/15	4/8	1.4/6	30/150	8/16	0.4/0.8	2/5	0.5/5
Imitation dairy			6/12						2/4	
Instant coffee/tea	0.1/1	0.005/0.01			0.5/3	2/10				
Jams/jellies	0.5/5				1.4/6	20/80				
Meat products	1.3/5				1.2/5				1/2	0.5/5
Milk products	0.3/3				0.8/4	5/50	4/8	0.2/0.4	1/2	0.1/1
Nut products										0.5/5
Other grains										
Poultry										0.03/0.3
Processed fruits										
Processed vegetables										0.1/1.0
Reconstituted vegetables										0.003/0.03
Seasonings/ flavors	0.5/5				1.4/6.0	5/30				0.5/5
Snack foods	0.5/5				1.4/6.0	5/30		0.2/0.4	1/2	1.0/5
Soft candy	1.0/4			3.0/5.0	1.4/6.0	30/150	6/12	0.3/0.6	2/4	
Soups	1.0/2.5			2.0/4.0	0.5/3.0	1/10	4/8	0.2/0.4	2/4	0.1/1.0
Sugar substitutes										
Sweet sauces										

Table 6 continued on page 50 ▶

GRAS Flavoring Substances 20

Table 6—Use levels for new FEMA GRAS flavoring substances *(continued)*

	Average usual ppm/Average maximum ppm										
	11	12	13	14	15	16	17	18	19	20	21
	Ethyl 4-(acetylthio) butyrate	Ethyl *cis*-4-hepte-noate	Ethyl 5-hexe-noate	(+/-) Ethyl 3-mercapto butyrate	Ethyl 5-(methylthio) valerate	Furfuryl propyl disulfide	(+/-) Heptan-3-yl acetate	(+/-) Heptan-2-yl butyrate	(Z)-3-Hexenyl (E)-2-butenoate	(E)-2-Hexenyl hexa-noate	4-Hydroxy benzal-dehyde
Category	**FEMA No. 3974**	**3975**	**3976**	**3977**	**3978**	**3979**	**3980**	**3981**	**3982**	**3983**	**3984**
Baked goods	5/10	15/30	4/8	0.5/1	1.5/3	0.5/1	8/16	5/10	2/5		5/30
Beverages (nonalcoholic)	2/4	8/12	3/5	0.2/0.4	0.5/1	0.2/0.4	5/10	2/4	2/4	0.5/5	1/10
Beverages (alcoholic)	2/4	8/12	3/5	0.2/0.4	0.5/1		5/10	2/4	0.5/3		3/20
Breakfast cereal									0.5/5		5/30
Cheese											5/30
Chewing gum	4/8	40/80	16/30	1/2	4/8	1/2	25/50	20/40	50/100		10/50
Condiments/ relishes	2/4			0.2/0.4	0.4/0.8	0.2/0.4					
Confectionery frostings									20/50		3/20
Egg products											3/20
Fats/oils											2/20
Fish products											
Frozen dairy	3/6	10/15	4/6	0.3/0.6	0.5/1	0.3/0.6	6/12	3/6	2/5		5/20
Fruit ices	3/6	8/12	3/5			0.2/0.4			1/5		2/20
Gelatins/ puddings	3/6		4/6	0.3/0.6	0.5/1	0.2/0.4			2/5		2/20
Granulated sugar	15/30								0.2/1		
Gravies				0.2/0.4	0.4/0.8	0.2/0.4			0.5/3		
Hard candy	2/4	12/20	4/8	0.4/0.8	1/2	0.4/0.8	7/14	4/8	20/50		5/30
Imitation dairy	3/6			0.2/0.4	0.3/0.6				2/5		
Instant coffee/tea				0.2/0.4							5/20
Jams/jellies									2/10		5/20
Meat products						0.4/0.8			0.5/8		
Milk products	2/4			0.2/0.4	0.5/1				0.5/2		3/20
Nut products									0.5/2		
Other grains											
Poultry											
Processed fruits									.5/2		
Processed vegetables									0.5/2		
Reconstituted vegetables											
Seasonings/ flavors									0.2/1		5/30
Snack foods	2/4				0.4/0.8	0.2/0.4	3/6				
Soft candy	3/6	10/15	4/6	0.3/0.6	0.5/1	0.3/0.6	7/14	3/6	5/10		5/20
Soups	3/6			0.2/0.4		0.4/0.8			0.5/2		
Sugar substitutes									0.5/2		
Sweet sauces									0.5/3		

Table 6 continued on page 51 ▶

Table 6—Use levels for new FEMA GRAS flavoring substances *(continued)*

	Average usual ppm/Average maximum ppm									
	22	23	24	25	26	27	28	29	30	31
	2-Hydroxy-benzoic acid	4-Hydroxy-benzoic acid	4-Hydroxy benzyl alcohol	4-Hydroxy-3-methoxy-benzoic acid	3(2) – Hydroxy-5-methyl -2(3)-hexanone	Isopentyl-idene isopentyl-amine	Isoprenyl acetate	d,l-Menthol (+/-)-propylene glycol carbonate	*erythro and threo*-3-Mercapto -2-methyl-butan -1-ol	3-Mercapto -2-methyl-pentanal
Category	**FEMA No. 3985**	**3986**	**3987**	**3988**	**3989**	**3990**	**3991**	**3992**	**3993**	**3994**
Baked goods		60/360				0.15/0.8	10/100	60/250	0.1/1	0.05/0.5
Beverages (nonalcoholic)	5/50	20/100	5/25		3/6	0.01/0.1	0.3/3	30/120		
Beverages (alcoholic)	10/100	50/300		5/25	3/8	0.05/0.3	1.5/15	100/400		
Breakfast cereal						0.01/0.05	0.3/1.5	15/60		0.03/3
Cheese								15/60		
Chewing gum						0.12/1	15/100	5000/20000		
Condiments/ relishes								100/400	0.1/1.0	0.03/0.3
Confectionery frostings				5/25		0.05/0.5	1.5/10	500/2000		
Egg products						0.05/0.5	1.5/10			
Fats/oils		30/200				0.09/0.8	1.5/10		0.1/0.5	0.05/0.5
Fish products										0.01/0.1
Frozen dairy	10/100	50/300	20/100	5/25	3/7	0.03/0.3	6/60	30/120		
Fruit ices	10/100	50/300	20/100			0.02/0.2	3/30	100/400		
Gelatins/ puddings					3.5/9	0.02/0.2	3/30	200/800		
Granulated sugar										
Gravies						0.05/0.5		25/100		0.05/0.5
Hard candy						0.10/0.7	5/50	500/2000		
Imitation dairy					3.5/6			15/60		
Instant coffee/tea						0.02/0.1	0.6/6	100/400		
Jams/jellies	10/100						3/30			
Meat products						0.05/0.5			0.1/2.0	0.03/0.3
Milk products	5/50	50/300	15/75	3/15	3.5/6	0.11/0.7	0.6/60	200/800		
Nut products										
Other grains										0.01/0.1
Poultry										
Processed fruits								100/400		
Processed vegetables										0.01/0.1
Reconstituted vegetables									0.01/0.1	
Seasonings/ flavors						0.1/0.5	1.5/15			0.03/1
Snack foods						0.1/0.5		25/100	0.1/0.5	0.01/0.1
Soft candy		50/300				0.13/0.6	3/30	500/2000		
Soups						0.01/0.08		25/100	0.1/1	0.03/0.3
Sugar substitutes										
Sweet sauces										

Table 6 continued on page 52 ▶

GRAS Flavoring Substances 20

Table 6—Use levels for new FEMA GRAS flavoring substances *(continued)*

	Average usual ppm/Average maximum ppm									
	32	33	34	35	36	37	38	39	40	41
	(+/-) 2-Mercapto-2-methyl pentan-1-ol	3-Mercapto-2-methyl-pentan-1-ol	4-Mercapto-4-methyl-2-pentanone	(+/-) – 2-Methyl-1-butanol	(+/-) 3-Methyl *gamma*-deca lactone	2-Methyl heptan-3-one	(E)-6-Methyl-3-hepten-2-one	Methyl 2-methyl-propenoate	Methyl (methyl thio) acetate	2-(Methyl thio) ethanol
Category	**FEMA No. 3995**	**3996**	**3997**	**3998**	**3999**	**4000**	**4001**	**4002**	**4003**	**4004**
Baked goods	0.005/0.05	0.005/0.05	5/10	2/9	1/3	12/25	3/6		4/8	8/16
Beverages (nonalcoholic)	0.002/0.05		0.1/1	0.1/2	0.3/1	7/15	1/1.5	2/10	2/4	3/6
Beverages (alcoholic)	0.002/0.05		0.5/5	0.2/4	0.5/1.5	5/10	1/1.5	4/20		
Breakfast cereal		0.003/0.03	0.1/0.5		1/3					
Cheese										
Chewing gum	0.01/0.2		10/30	2/15	2/4	25/40			8/16	
Condiments/ relishes	0.01/0.2	0.003/0.03							2/4	3/6
Confectionery frostings	0.005/0.05		0.5/5	0.3/2	1/2					
Egg products				0.3/2						
Fats/oils	0.005/0.05	0.005/0.05		0.5/15				4/20		
Fish products		0.001/0.01								
Frozen dairy	0.004/0.04		2/20	1.5/8	1/2	10/20	2/4		2/4	5/10
Fruit ices	0.004/0.04		1/10	0.8/5	0.5/1.5			2/10		
Gelatins/ puddings	0.004/0.04		0.5/5	0.2/2	0.5/1.5					
Granulated sugar										
Gravies	0.01/0.1	0.005/0.05		0.3/2.5					2/4	4/8
Hard candy	0.005/0.1		5/25	0.3/3	0.5/1.5	12/24	2/4		4/8	6/10
Imitation dairy						8/16	1/2		2/4	3/6
Instant coffee/tea			0.2/2	0.1/1						
Jams/jellies			1/5	0.4/4	1/3					
Meat products	0.01/0.1	0.003/0.03							2/4	3/6
Milk products			0.1/1	0.1/1	0.5/1.5			2/10		3/6
Nut products										
Other grains		0.001/0.01								
Poultry										
Processed fruits										
Processed vegetables		0.001/0.01								
Reconstituted vegetables		0.001/0.01								
Seasonings/ flavors		0.03/10000		0.3/2.5						
Snack foods		0.001/0.01		0.4/4					2/4	4/8
Soft candy	0.005/0.1		1/5	0.4/4	1/3		1/2		2/4	3/6
Soups	0.004/0.1	0.003/0.03		0.2/2					2/4	4/8
Sugar substitutes										
Sweet sauces										

Table 6 continued on page 53 ▶

Table 6—Use levels for new FEMA GRAS flavoring substances *(continued)*

	Average usual ppm/Average maximum ppm									
	42	43	44	45	46	47	48	49	50	51
	12-Methyl tridecanal	L-Mono-menthyl glutarate	(+/-) Nonan-3-yl acetate	(E,E)-3,5-Octadien-2-one	(+/-) Octan-3-yl formate	Par-aldehyde	4-Pentenyl acetate	2-Pentyl acetate	Perilla Leaf Oil	Phenethyl isothio cyanate
Category	**FEMA No. 4005**	**4006**	**4007**	**4008**	**4009**	**4010**	**4011**	**4012**	**4013**	**4014**
Baked goods	35/70		9/18	4/8	10/20	80/200	20/40	30/120	10/100	8/80
Beverages (nonalcoholic)	0.7/7	50/125	6/12	2/5	6/10	3/20	8/16	10/20	2/200	0.15/4
Beverages (alcoholic)		50/150	6/12	2/5		15/40	8/16	10/25	1/10	0.75/7.5
Breakfast cereal	0.7/3.5					3/12		1/10		
Cheese										0.18/1.8
Chewing gum		1500/4000		16/30	30/50	80/200	100/200	125/300	20/2000	8/80
Condiments/ relishes										
Confectionery frostings		200/600				15/100		5/25	1/20	0.75/7.5
Egg products	3.5/35					15/100		5/25		0.75/7.5
Fats/oils	3.5/35					15/100		5/25	1/10	0.75/7.5
Fish products										0.75/7.5
Frozen dairy			7/14	2/5	8/12	10/60	16/32	15/30	2/200	1.5/15
Fruit ices						8/40		1.5/8	2/200	
Gelatins/ puddings						10/50		1.5/8	2/200	1.5/15
Granulated sugar										
Gravies	3.5/35							3/15		0.75/7.5
Hard candy	3.5/35	300/700	8/16	4/8	10/20	20/89	20/50	25/60	2/200	1.5/20
Imitation dairy				2/4	8/12			10/20		1/10
Instant coffee/tea						5/30		1.5/8		
Jams/jellies								5/25		1.5/15
Meat products	3.5/35									0.75/7.5
Milk products	0.7/7					5/40	10/20	10/20		0.3/3.0
Nut products										
Other grains										
Poultry										
Processed fruits										
Processed vegetables									5/100	
Reconstituted vegetables										
Seasonings/ flavors	3.5/35					15/50		5/25	100/500	8/50
Snack foods	7/35	40/80		2/4				5/25	1/100	1.5/15
Soft candy		250/600	7/14	3/6	8/12	20/60		5/25	2/200	1.5/15
Soups	0.7/7							1/10	1/100	0.15/1.5
Sugar substitutes										
Sweet sauces										

Table 6 continued on page 54 ▶

GRAS Flavoring Substances 20

Table 6—Use levels for new FEMA GRAS flavoring substances *(continued)*

	Average usual ppm/Average maximum ppm								
	52	53	54	55	56	57	58	59	60
	Pyrazine	Sodium 4-methoxy benzoyl oxyacetate	2,4,6-Triisobutyl-5,6-dihydro-4H-1,3,5-dithiazine	2,4,6-Trimethyl-dihydro-4H-1,3,5-dithiazine	3,7,11-Trimehtyl-2,6,10-dodeca trienal	(+/-)-(2,6,6-Trimethyl-2-hydroxycyclo-hexylidene) acetic acid gamma lactone	2,3,5-Trithia-hexame	6-Un-decanone	Vanillin *erythro and threo*-butan-2,3-diol acetal
Category	FEMA No. 4015	4016	4017	4018	4019	4020	4021	4022	4023
Baked goods	1/5	80/200	0.2/2	8/16	2/10		2/10	8/15	200/400
Beverages (nonalcoholic)	0.3/1.5			3/6	0.1/0.8		0.1/0.8	5/10	60/120
Beverages (alcoholic)	0.6/3				0.3/2		0.3/2		60/120
Breakfast cereal		200/600	0.1/1	0.07/0.35	0.1/0.8				180/300
Cheese									
Chewing gum				10/20	2/15		2/10		250/500
Condiments/ relishes			0.1/1	3/6					60/120
Confectionery frostings	0.6/3	300/600			0.3/2		0.3/2		
Egg products				4/8	0.3/2		0.3/2		
Fats/oils			0.2/2	0.35/3.5	0.3/2		0.3/2		
Fish products			0.04/0.4	0.35/3.5					
Frozen dairy	0.6/3			4/8	0.5/6		0.5/6	7/10	80/160
Fruit ices				3/6	0.2/2.1		0.2/1		
Gelatins/ puddings				3/6	0.2/2		0.2/1		100/200
Granulated sugar									
Gravies			0.2/2	4/8	0.3/2		0.3/2		
Hard candy	1/5			5/10	0.5/5		0.5/2	8/15	150/280
Imitation dairy				3/6				5/10	60/120
Instant coffee/tea					0.1/1		0.1/0.8		
Jams/jellies		400/1000			0.5/5		0.5/3		
Meat products			0.1/1	3/6			0.4/5		
Milk products	0.3/1.5			3/6	0.2/2.1		0.2/1		60/120
Nut products									60/120
Other grains			0.04/0.4						
Poultry				0.2/2					
Processed fruits		100/300							
Processed vegetables			0.04/0.4	0.2/2					
Reconstituted vegetables			0.04/0.4	0.2/2					
Seasonings/ flavors				200/500	0.5/5	1.5/1.5	0.5/5		
Snack foods		300/500	0.04/0.4	3/6	0.5/5		0.5/3		60/120
Soft candy	1/5	30/100		4/8	0.5/5		0.5/3	7/10	120/240
Soups			0.1/1	4/8	0.1/0.8		0.1/1		60/120
Sugar substitutes									
Sweet sauces		500/900							

Table 7—Updated use levels for flavoring substances previously recognized as FEMA GRAS, on which the FEMA Expert Panel based its judgments that the substances are generally recognized as safe (GRAS). Superscript a represents a new use level

	Average usual ppm/Average maximum ppm						
	Cyclopentanone	2,4-Hexadien-1-ol	(Z)-3-Hexenyl (E)-2-methyl-2-butenoate	(E)-2-Hexenyl butyrate	(Z)-3-Hexenyl isobutyrate	(Z)-3-Hexenyl valerate	Neohesperidin dihydrochalcone
Category	FEMA No. 3910	3922	3931	3926	3929	3936	3811
	GRAS List 19	19	19	19	19	19	17
Baked goods	2/3		16/30	7.2/40[a]	16/150[a]	15/30[a]	4/4
Beverages (nonalcoholic)	1/15[a]	1/6	2/5[a]	5/10[a]	2/20[a]	0.5/3[a]	2/3
Beverages (alcoholic)		0.5/1	4.1/8.1	5/10[a]	4/8	4/8	3/3
Breakfast cereal	1/2						3/3
Cheese							3/4
Chewing gum			0.1/0.1	20/50[a]	15/150[a]	5/30[a]	200/200[a]
Condiments/relishes	1/2	2/4			5/50[a]		2/3
Confectionery frostings		0.1/1[a]		10/20[a]	5/50[a]		3/3
Egg products							2/3
Fats/Oils							4/4
Fish products							2/3
Frozen dairy		2/4	7/15	5/20[a]	7/15	7/15	2/3
Fruit ices							1/2
Fruit juices					4/8	4/8	
Gelatins/puddings		0.7/2	4/8	5/20[a]			2/3
Gravies		0.073/0.073					3/4
Hard candy		0.3/3[a]	0.2/0.2	10/20[a]	5/50[a]	3/18[a]	2/4
Ice cream/ices							
Imitation dairy				5[a]			3/4
Instant coffee/tea							2/3
Jams/jellies		1/2	2/4	0.5/1			2/3
Meat products							2/3
Milk products			0.5/5[a]	5/20[a]	2/20[a]	0.5/3[a]	2/3
Nut products							3/4
Other grains							3/4
Poultry							2/3
Processed fruits							2/3
Processed vegetables							2/3
Reconstituted vegetables							2/3
Seasonings/Flavors		0.1/0.5					3/4
Snack foods		0.4/0.4					3/3
Soft candy		1/2	2/3	10/20[a]			2/3
Soups	1/2	0.13/0.13					1/2
Sugar substitutes							4/4
Sweet sauces	1/2			0.5/1			2/3

仮 訳

香料化合物の摂取量／暴露量の推定

（抜粋）

Estimation of Intake/Exposure to Flavoring Substances

[GRAS Flavoring Substances 20 (FOOD TECHNOLOGY、December 2001、Vol.55、No.12) より抜粋]

香料化合物の摂取量／暴露量の推定

食品の技術進歩に伴い、それが人類の食事に与える影響はさらに明確なものになってきている。グローバルな食品供給は、その量や質に伴って発展し、またこの分野の科学的進歩によって創られた健康的で栄養のある食品の多様性により発展してきた。食品の質および量を支え、かつその発展にあたり、製造業者による保存料、色素、添加物及び食品香料の使用は重要な役割を果たしている。これらの直接的な進歩の成果として、食品添加物、特に食品香料が人の消費に対して安全であることを、規制当局や消費者に保証するために数多くの安全性評価方法が開発され、現在利用されている点である（JECFA, 1968,1996,1998,1999,2000；NAS, 1970,1980；Oser and Hall, 1977, FSC, 1980；FDA, 1982,1993；WHO, 1987；SCF, 1991；Hallagan and Hall, 1995；Munro et al., 1999)。

香料化合物の暴露量もしくは摂取量は、その物質を経口で摂取した量と定義され、食品成分の安全性を評価する上で不可欠である。香料化合物の摂取量を定量化することは、気力がくじけるような困難な仕事であり、技術的および経済的に困難な多くの問題が待ち受けている。例えば、西部地区の食生活では 20,000 種類以上のさまざまな食品が購入可能であるが(FMI,1998)、これらの製品は不定期に数多くのさまざまな人々によって消費されるため、ある食品成分について各個人の摂取量を決定することが困難である。このことに加え、様々な人々の摂食者（eaters）に対して統計学的に有意な結果を得るのに充分な大きさの集団の詳細な食事分析が必要となるため、正確な摂取量データを得ることの難しさと経費の高さが問題である。

40 年以上もの間、政府の役人、科学者及び食品工業の専門家は、食品中の香料化合物の暴露量を推定するための様々な方法を提案してきた。初期の頃は、暴露量は PADI (possible average daily intake =可能平均一日摂取量）と呼ばれる方法を用いて算出した。PADI 法は、食品に添加される香料化合物の使用量とその食品が消費された量に基礎をおく方法である（下記参照)。残念ながら、この方法は人の消費パターンに係わる複雑さと食品供給量とを結びつけることができず、一般に摂取量は非常に過大な評価結果となる。

現在、香料化合物の暴露量を決定するために用いられている方法は、フレーバーとして使用される物質について、米国においては推定 PADI 法であり、ヨーロッパでは TAMDI 法（theoretical added maximum daily intake = 理論的最大添加一日摂取量)である。この PADI 法は、(1) 33 種の各食品カテゴリー(例えば焼成品や肉製品)中の物質の通常使用率に、そのカテゴリー食品の一日平均消費量を乗じ、(2) 次に、全ての 33 カテゴリーについての摂取量を合計することにより決定される（USDA/ARS、1973)。

年間使用量が低いと報告されてきたフレーバーの殆どは（Lucas et al., 1999；IOFI, 1995)、この PADI 法による一日平均摂取量の総量では過大評価されたものとなる。この PADI 法による計算は、ある食品カテゴリーにおける全ての食品が常にその物質を含んでいると仮定し、さらに、そのカテゴリーの食品が毎日消費されるものと仮定する（Oser& Hall, 1977)。この仮定がどのような問題を引き起こすかという事例として、Ethyl methyl phenyl glycidate をとりあげる。この物質は、ストロベリーフレーバーの一部としてハードキャンディーに添加されるが故に、PADI 法による計算では、この物質が全てのハード