Recent Progress in the Consideration of Flavoring Ingredients Under the Food Additives Amendment

15. GRAS Substances

A list of flavoring ingredient substances considered generally recognized as safe by the Flavor & Extract Manufacturers' Association Expert Panel

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☐ The Flavor and Extract Manufacturers' Association (FEMA) Expert Panel was formed in response to the provision in the 1958 Food Additive Amendment that exempted those substances Generally Recognized As Safe (GRAS) from food additive status. According to the amendment, GRAS status is conferred by recognition among ". . . experts qualified by scientific training and experience to evaluate its [the substance's] safety . . . under the conditions of its intended use." (CFR, 1988)

Areas of expertise embraced in the current Panel membership include toxicology, biostatistics, metabolism and pharmacokinetics, biological chemistry, pathology, nutrition, organic chemistry, and related fields. Consultants in additional areas of expertise are sought as needed on an *ad hoc* basis.

The FEMA Expert Panel has changed little in size from the original seven members organized by Dr. Bernard Oser in 1960. The current membership and their affiliations are Lauren A. Woods, Ph.D., M.D., Professor Emeritus, Medical College of Virginia, Virginia Commonwealth University; John Doull, M.D., Ph.D., Professor, University of Kansas Medical School; Paul M. Newberne, D.V.M., Ph.D., Professor of Pathology, Boston University School of Medicine; Carrol S. Weil, M.A., Carrol S. Weil, Inc.; Robert L. Smith, Ph.D., D.Sc., Professor, St. Mary's Hospital, University of London; Bernard M. Wagner; M.D., Associate Director, Nathan Kline Neurological Institute, Orangeburg, NY; Philip S. Portoghese, Ph.D., Professor, University of Minnesota; Ian C. Munro, Ph.D., Director, Canadian Centre for Toxicology, Guelph, Ontario.

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Dr. Woods served as chairman of the panel, *pro tempore,* from July 1984 to July 1985 and Mr. Weil served in a similar capacity from July 1985 to July 1986. Dr. Paul Newberne was elected Chairman in July 1986.

Expert Panel founder and Chairman Emeritus, Dr. Bernard L. Oser has retired from active Panelist status. As the first Honorary member, Dr. Oser consults with the Panel as requested.

Dr. Richard A. Ford serves as liaison expert with the Research Institute for Fragrance Materials Expert Panel. Dr. George A. Burdock has assumed the duties of the FEMA Director of Scientific Affairs and Executive Secretary of the Expert Panel.

Objective of the Expert Panel

Although the evaluation of the GRAS status of flavoring materials is the function for which the Panel is most noted, the principal objective of the Expert Panel in the context of the Federal Food Drug and Cosmetic Act (CFR, 1989):

> To protect the consumer's health in the context of flavor use and to provide the scientific basis for helping industry maintain a pattern of self-regulation (FEMA Expert Panel, 1986).

Evaluation of a flavor ingredient proposed for GRAS status begins with the submission of a completed GRAS application form including a complete literature search. The FEMA staff provides a preliminary assessment of the data for adequacy and prepares a monograph on the candidate substance and structurally related materials. The application and supplemental material are then reviewed by the Panel at the next regularly scheduled meeting. The review process involves the application of a minimum base-set of evaluative criteria. Each criterion is considered and none is employed to the exclusion of another; all must be mutually supportive. Briefly, the criteria are chemical identity, purity, structural analogy, natural occurrence in food, concentration of the chemical in food and in the total diet, toxicity and metabolism in animals, and where possible, metabolism in man (Oser and Hall, 1977).

Following review of the material, the Panel may make one of the following decisions: GRAS, not GRAS, or in-

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sufficient data for determination of GRAS; in which case, the substance is placed in HOLD status pending submission of additional information. Those substances found to be GRAS are published in this journal along with the conditions of intended use as flavor ingredients (see References for a complete list of GRAS publications).

Review of existing GRAS substances is an equally important function of the Expert Panel. The Panel is now embarking on the second review cycle. In this review process, the Panel evaluates all data, both original data contributing to the first GRAS evaluation and any new data, relevant to the safety-in-use of existing flavor materials.

The selection of candidate flavors for review are made using the Ad Hoc Priority Setting Group algorithm (Easterday *et al.*, 1984). Criteria for the algorithm include structural analogy and consumption of the substance both as naturally present and as added to food. Summaries of those materials and structurally related flavor substances identified by the algorithm are reviewed by the Panel, and a decision is made regarding the continued GRAS status of the substances.

From time to time, the Expert Panel conducts detailed evaluations of previously GRASed flavor ingredients not selected by the priority system. This may occur as an outgrowth of the review of other GRAS substances or as the result of newly developed data. On the basis of the information developed in the studies, the substance may remain GRAS, (e.g. caffeine, cinnamyl anthranilate) it may have its GRAS status removed (e.g. calamus oil, brominated vegetable oil) or it may be placed in the HOLD category pending additional information.

Recognition of the work accomplished by the Panel is best judged by peers following publication in journals of appropriate readership. Pursuant to this, the Panel has emphasized the need to publish findings resulting from Panel initiated studies. FEMA is responding by preparing this data for publication. The Panel has in the past and will continue to supply the Food and Drug Administration pre-publication copies of each GRAS list and summaries of the data supporting each evaluation.

Lastly, the Panel is aware of the necessity to pursue new approaches to safety evaluation of flavors commensurate with evolving safety evaluation procedures. In terms of approaches, the Panel is evaluating the role of short-term testing and other *in vitro* methods, metabolic mechanisms, and is placing increased emphasis on human metabolic studies. Developments in this area include adoption of a Primary Toxicity Screen for test substances. The Primary Toxicity Screen calls for repeated dosing of a substance to the test animal as opposed to the outmoded single-dose LD_{50} which was popular a few years ago.

Much of the progress in animal testing has been made through an understanding of the metabolic and pharmacokinetic processes in animals and man. Many of the early successes in this area were fostered by the research group at St. Mary's Hospital Medical School at the University of London. FEMA currently supports a substantial research program at St. Mary's and has expanded their work on estragole-like substances. This program also includes work on the cinnamyl esters and related substances. Information derived from this program has aided in the prediction of the metabolism of newly proposed flavor ingredients.

Safety Assessment of D-Limonene

The National Toxicology Program, in its draft report on the bioassay of d-limonene (FEMA No. 2633) concluded:

"Under the conditions of these 2-year gavage studies, there was clear evidence of carcinogenic activity of d-limonene for male F344/N rats, as shown by increased incidences of tubular cell hyperplasia, adenomas and adenocarcinomas of the kidney. There was no evidence of carcinogenic activity of d-limonene for female F344/N rats that received 300 or 600 mg/kg. There was no evidence of carcinogenic activity of d-limonene for male $B6C3F_1$ mice (250 or 500 mg/kg) or for female $B6C3F_1$ mice at 500 or 1,000 mg/kg." (NIH, 1988)

Thus the "clear evidence" was based exclusively on the male rat response. This unique response has now been studied in great detail and the essential mechanisms have been identified.

Pathogenesis of d-Limonene Nephrotoxicity Culminating in Carcinoma. The male rat produces a sex-specific quantity of a low molecular weight protein termed alpha 2μ-globulin. This protein is produced in the liver under the influence of testosterone and is readily filtered through the glomeruli of the kidney. There is now abundant data to demonstrate that the kidney lesions (hyaline droplets or enlarged phagolysosomes, tubular cell degeneration, granular casts) induced in male rats given d-limonene for 13 weeks is related to the accumulation of alpha 2μ-globulin within the epithelial cells of the P2 segment of the nephron.

At 24 hours after oral administration of d-limonene, the renal concentration is approximately 2.5 times higher in male rats than in female rats. Also, in male rat kidney, about 40% of the administered d-limonene equivalents is associated with proteins in a reversible manner. This is not observed in female rat studies. It appears that d-limonene associated with a protein present only in male rat kidney; this protein was clearly identified as alpha 2μ-globulin by amino acid sequencing. The major metabolite associated with this globulin was d-limonene-1,2-oxide. Parent d-limonene was also identified as a minor component in the alpha 2μ-globulin fraction. The demonstration that d-limonene, and more specifically d-limonene-1,2-oxide, associated with this globulin in a reversible manner in male rat kidney accounts for the excessive accumulation of alpha 2μ-globulin in male rats administered d-limonene.

As predicted by 13-week studies, the kidney of male rats is the target organ in the 2-year study. Proliferative lesions were induced ranging from tubular cell hyperplasia and adenomas to adenocarcinomas. This is in keeping with contemporary biological concepts relating sustained cell injury and degeneration with necrosis to increased cell replication rates promoting spontaneously initiated epithelial cells. Cell degeneration and necrosis in the P2 segment of the proximal convoluted tubules associated with the accumulation of alpha 2μ-globulin have been demonstrated for other compounds as well. (Table 1)

While humans produce low molecular weight serum proteins which are reabsorbed by the kidney, there is no evidence that alpha 2μ-globulin is produced. It is not known if any human serum proteins possess a binding site similar to that of alpha 2μ-globulin. While this is a possibility, it appears remote; since female rats, mice,

and dogs do not show the renal changes noted in male rats exposed to d-limonene. The accumulated evidence points to the unique anatomical, physiological, and biochemical properties of the male rat kidney, especially the proximal convoluted tubule. The conclusion appears to be that d-limonene disturbs the renal processing of alpha 2μ-globulin, unique to male rat kidney, and is not predictive for humans. Data show that the tumor results in the male rat are inseparable from the toxicologic response.

Based strictly on empirical data alone, the NTP 2-year gavage studies of d-limonene did produce "clear evidence" of carcinogenicity for male F-344/N rats. However, the mechanisms leading to this end result are largely known and are extremely persuasive that the NTP results have no significance for humans. The FEMA Expert Panel after careful review of all the toxicology, pathology, and related studies, do not find that d-limonene presents a risk for human use. Accordingly, the Panel recommends that under the conditions of intended use as a flavoring substance, d-limonene is generally recognized as safe for humans.

Notes to the Reader

In response to questions regarding the definition of certain materials, the Panel has defined substances as follows:

- **Amyl/isoamyl alcohol:** Amyl or isoamyl alcohol is a product consisting of one or a mixture of isomers of primary amyl alcohols.
- **Valeric/isovaleric acids and esters:** Valeric acid/isovaleric acid and their esters are products consisting of one or a mixture of isomers of *n*-pentenoic acid and/or 2- or 3-methyl butanoic acid.
- **Isovaleraldehydes:** The Panel declined to take further action in the matter of the aldehydic forms of valeric/isovaleric acid. There is no need for an extended definition as the result of the broadness of the specifications for valeraldehyde, 2-methylbutyraldehyde and 3-methylbutyraldehyde, all of which are considered GRAS (FEMA Nos. 3098, 2691, and 2692, respectively).
- **Glycine (FEMA No. 3287):** The use level for glycine in Beverage Type I was increased from a maximum of 150 ppm to 1000 ppm.

Table 1—**Compounds** *which produce alpha 2μ-globulin nephropathy/tumors in male rats*

Jet fuels	Unleaded gasoline
Isoparaffine	Paradichlorobenzene
Hexachloroethane	Dimethyl methylphosphorate
Isophorone	Pentachloroethane
d-Limonene	Decalin

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—*"Primary Names and Synonyms Alphabetical Cross Reference List" and "GRAS Flavoring Ingredients and Usage Levels" start on p. 84*

GRAS 15—Primary Names and Synonyms[a] Alphabetical Cross Reference List

FEMA No.	Substance
3755	DEHYDROMENTHOFUROLACTONE 2(4H)-Benzofuranone, 5,6-dihydro-3,6-dimethyl-, (R)- 3,6-Dimethyl-5,6-dihydro-2(4H)benzofuranone 3,6-Dimethyl-4,5-dihydro-6H-benzo(b) furan-2-one
3756	4-ETHYLBENZALDEHYDE Benzaldehyde, 4-ethyl p-Ethylbenzaldehyde
3757	ETHYL METHYL-P-TOLYLGLYCIDATE Ethyl methyl-p-methylphenylglycidate Oxiranecarboxylic acid, 3-methyl-3-(4-methylphenyl)-ethyl ester
3758	5-HYDROXY-8-UNDECENOIC ACID DELTA-LACTONE Jasmolactone extra C 2H-Pyran-2-one, 6-(3-hexenyl)tetrahydro-, (Z)-
3759	5-ISOPROPENYL-2-METHYL-2-VINYLTETRAHYDROFURAN Anhydro linalool oxide 2-Ethenyl-2-methyl-5-(1-methylethenyl) tetrahydrofuran Furan, 2-ethenyl-tetrahydro-2-methyl-5-(1-methylethenyl)- 2-Methyl-2-vinyl-5-isopropenyltetrahydrofuran
3760	1-(4-METHOXYPHENYL)-4-METHYL-1-PENTEN-3-ONE alpha-p-Dimethylanisalacetone Isopropyl 4-methoxystyryl ketone Methoxystyryl isopropyl ketone 1-Penten-3-one, 1-(4-methoxyphenyl)-4-methyl-
3761	5-METHYL-2-HEPT-4-ONE Filbertone Hazeltone 2-Hepten-4-one, 5-methyl-
3762	3-METHYL-1-PENTANOL 2-Ethyl-4-butanol 1-Pentanol, 3-methyl-
3763	3-METHYL-2-(N-PENTANYL)-2-CYCLOPENTEN-1-ONE 2-Cyclopenten-1-one, 3-methyl-2-pentyl- Dihydrojasmone 2-Pentyl-3-methyl-2-cyclopenten-1-one
3764	MINTLACTONE 2(4H)-Benzofuranone,5,6,7,7a-tetrahydro-3,6-dimethyl- Dehydroxymenthofurolactone 3,6-Dimethyl-5,6,7,7a-tetrahydro-2(4H) benzofuranone 3,6-Dionethyl-4,5,6,7-tetrahydro-7aH-benzo(b) furan-2-one
3765	MYRTENYL ACETATE Bicyclo[3.1.1]hept-2-ene-21-methanol, 6-6-dimethyl-,acetate, (1S)- 2,2-Pinene-10-yl-acetate 2-Pinen-10-ol acetate
3766	2-TRANS-6-TRANS-NONADIENAL 2,6-Nonadienal, (E,E)-
3767	3-OXODECANOIC ACID GLYCERIDE Glyceryl ester of 3-oxodecanoic acid
3768	3-OXODODECANOIC ACID GLYCERIDE Glyceryl ester of 3-oxododecanoic acid
3769	3-OXOHEXADECANOIC ACID GLYCERIDE Glyceryl ester of 3-oxohexadecanoic acid
3770	3-OXOHEXANOIC ACID DIGLYCERIDE Glyceryl ester of 3-oxohexanoic acid
3771	3-OXOOCTANOIC ACID GLYCERIDE Glyceryl ester of 3-oxooctanoic acid
3772	3-OXOTETRADECANOIC ACID GLYCERIDE Glyceryl ester of 3-oxotetradecanoic acid
3773	SODIUM 2-(4-METHOXY PHENOXY)PROPANOATE Propanoic acid, 2-(4-methoxyphenoxy),sodium salt
3774	THEASPIRANE 1-Oxaspiro[4.5]dec-6-ene, 2,6,10,10-tetramethyl- 1-Oxaspiro-2,6,10,10-tetramethyl[4.5]dec-6-ene 2,6,10,10-Tetramethyl-1-oxaspiro[4.5]dec-6-ene

[a] Primary names, in capital letters, and synonyms, in lower case, are listed alphabetically. Synonyms are followed by reference to the primary name and FEMA number.

GRAS Flavoring Ingredients and Usage Levels

Flavor and Extract Manufacturers' Association average maximum levels (in ppm) on which the Expert Panel based its judgments that the substances are generally recognized as safe for their intended uses

FEMA No.	Substance	Baked Goods	Frozen Dairy	Meat Products	Soft Candy	Gelatins & Puddings	Soups	Snack Foods	Nonalcoholic Beverages	Alcoholic Beverages	Other Uses
3755	Dehydromenthofurolactone	—	—	—	—	2	—	—	1	—	Hard candy—5.0; Chewing gum—20.0; Confectionary & frosting—5.
3756	4-Ethylbenzaldehyde	25	15	5	40	25	—	—	15	15	Breakfast cereals—25; Dairy products—15; Fruit ices—10; Poultry—5; Jams & jellies—40; Imitation dairy products—15; Chewing gum—200; Instant coffee & tea—15; Condiments/relishes—10; Confectionary & frosting—25; Seasonings & flavorings—15; Nut products—25; Hard candy—40

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GRAS Flavoring Ingredients and Usage Levels, (continued)

FEMA No.	Substance	Baked Goods	Frozen Dairy	Meat Products	Soft Candy	Gelatins & Puddings	Soups	Snack Foods	Nonalcoholic Beverages	Alcoholic Beverages	Other Uses
3757	Ethyl methyl-p-tolylglycidate	17.5	14	—	16	15.6	—	—	10.4	10	
3758	5-Hydroxy-8-undecenoic acid delta-lactone	3	10	—	1.5	3	—	—	3	5	Dairy products—2; Confectionary & frosting—3; Sweet sauce—3; Hard candy—10; Fruit ices—0.1; Jams & jellies—3; Imitation dairy products—1; Chewing gum—15
3759	5-Isopropenyl-2-methyl-2-vinyltetrahydrofuran	—	0.1	—	—	0.1	—	—	0.02	—	Dairy products—0.1; Fruit ices—0.12; Jams & jellies—0.12; Hard candy—0.12; Chewing gum—.09
3760	1-(4-Methoxyphenyl)-4-methyl-1-penten-3-one	10	—	—	—	—	—	—	—	—	
3761	5-Methyl-2-hept-4-one	20	5	—	20	20	5	20	0.5	0.5	Bread & cereals—20; Fats & oils—20; Dairy products—10; Processed fruit—5; Jams & jellies—5; Nut products—20; Imitation dairy products—10; Hard candy—20; Fruit ices—5; Confectionary & frosting—20; Chewing gum—50
3762	3-Methyl-1-pentanol	—	9	—	—	10	—	—	2	—	Dairy products—9; Fruit ices—8; Jams & jellies—10; Hard candy—10; Chewing gum—50;
3763	3-Methyl-2-(n-pentanyl)-2-cyclopenten-1-one	—	10	—	—	10	—	—	2	—	Fruit ices—13; Jams & jellies—13; Dairy products—10; Hard candy—13; Chewing gum—85
3764	Mintlactone	—	—	—	—	2	—	—	1	—	Confectionary & frosting—5; Chewing gum—20; Hard candy—5;
3765	Myrtenyl acetate	—	—	—	10	—	—	—	—	—	Confectionary & frosting—15; Hard candy—30; Chewing gum—31
3766	2-trans, 6-trans-Nonadienal	0.02	0.05	—	—	—	—	—	—	—	Fats & oils—0.2; Gravies—0.2; Imitation dairy products—0.2
3767	3-Oxodecanoic acid glyceride	10	—	—	—	—	—	—	—	—	Imitation dairy products—50
3768	3-Oxododecanoic acid glyceride	10	—	—	—	—	—	—	—	—	Imitation dairy products—50
3769	3-Oxohexadecanoic acid glyceride	20	—	—	—	—	—	—	—	—	Imitation dairy products—75
3770	3-Oxohexanoic acid diglyceride	5	—	—	—	—	—	—	—	—	Imitation dairy products—25
3771	3-Oxooctanoic acid glyceride	10	—	—	—	—	—	—	—	—	Imitation dairy products—50
3772	3-Oxotetradecanoic acid glyceride	10	—	—	—	—	—	—	—	—	Imitation dairy products—50
3773	Sodium 2-(4-methoxy phenoxy) propanoate	—	—	—	150	—	—	150	—	—	Confectionary & frosting—100
3774	Theaspirane	—	2.5	—	—	3	—	—	0.5	—	Dairy products—1.5; Fruit ices—1.8; Jams & jellies—2.5; Hard candy—3; Chewing gum—20