The FEMA GRAS Assessment of Alicyclic Substances Used as Flavour Ingredients

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Summary—For over 35 years, an independent panel of expert scientists has served as the primary body for evaluating the safety of flavour ingredients. This group, the Expert Panel of the Flavor and Extract Manufacturers' Association (FEMA), has achieved international recognition from the flavour industry, government regulatory bodies including the Food and Drug Administration, and the toxicology community for its unique contributions. To date, the Expert Panel has evaluated the safety of more than 1700 flavour ingredients and determined the vast majority to be "generally recognized as safe" (GRAS). Elements that are fundamental to the safety evaluation of flavour ingredients include exposure, structural analogy, metabolism, pharmacokinetics and toxicology. Flavour ingredients are evaluated individually taking into account the available scientific information on the group of structurally related substances. The elements of the GRAS assessment program as they have been applied by the Expert Panel to the group of 119 alicyclic substances used as flavour ingredients, and the relevant scientific data which provide the basis for the GRAS status of these substances, are described herein. This publication is the first of a series on GRAS flavour ingredients. Copyright © 1996 Published by Elsevier Science Ltd

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Abbreviations: ALT = alanine aminotransferase; CHO = Chinese hamster ovary; CYP450 = cytochrome *P*-450; FDA = Food and Drug Administration; FEMA = Flavor and Extract Manufacturers' Association; FFDCA = Federal Food, Drug and Cosmetic Act; SLR = Scientific Literature Reviews; GRAS = generally recognized as safe; GRASr = generally recognized as safe reaffirmation; LD_{50} = median lethal dose; MITO = mean intakes from total observations; NADPH = reduced nicotinamide adenine dinucleotide phosphate; NOAEL = no-observed-adverse-effect level(s); NTP = National Toxicology Program; OTC = over-the-counter; PADI = possible average daily intake; SCE = sister chromatid exchange(s).

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PART 1—THE FEMA GRAS ASSESSMENT PROGRAM

I. Introduction

The Food Additives Amendment to the Federal Food, Drug, and Cosmetic Act (FFDCA) provides at FFDCA Sec. 201(s) the authority for the determination of food ingredients as substances "generally recognized as safe" (GRAS).

> The term 'food additive' means any substance, the intended use of which results or may reasonably be expected to result, directly or indirectly, in its becoming a component or otherwise affecting the characteristics of any food ... if such substance is not generally recognized, among experts qualified by scientific training and experience to evaluate its safety, as having been adequately shown through scientific procedures (or, in the case of a substance used in food prior to January 1, 1958, through either scientific procedures or experience based on common use in food) to be safe under the conditions of its intended use ...

The definition removes GRAS substances from consideration as food additives, thereby explicitly excluding them from mandatory premarket approval by the US Food and Drug Administration (FDA). However, in order to be considered GRAS, substances must meet the series of strict criteria specified by Congress in the definition of food additive at FFDCA Sec. 201(s) (Hallagan and Hall, 1995).

In 1960, the Flavor and Extract Manufacturers' Association (FEMA) created a program to evaluate the safety of flavour ingredients and assess their status as GRAS under conditions of intended use. A key element of this program is the role of the Expert Panel comprising prominent experts in the fields of toxicology, chemistry, pharmacology, metabolism, pathology and statistics. For over 35 years the FEMA Expert Panel has evaluated more than 1700 flavour ingredients and assessed their status as GRAS. GRAS assessments are performed consistently according to well-defined criteria based on state-of-the-art science (Oser and Ford, 1991; Woods and Doull, 1991). Each Panel member applies these criteria and other relevant factors, using their individual scientific training and experience, to arrive at the decision. In order for a flavour ingredient to be considered GRAS, the decision must be unanimous. The history and operations of the FEMA Expert Panel have been reviewed (Oser and Ford, 1991).

The FEMA GRAS assessment program is an open process in which Panel decisions on individual flavour ingredients are provided to the FDA and are published on a regular basis. FEMA GRAS flavour ingredients are described in 14 publications in *Food Technology*, most recently in 1993 (Smith and Ford, 1993). The scientific data related to the GRAS assessment program were originally compiled in Scientific Literature Reviews (SLRs) by FEMA under a contract with the FDA. The SLRs are available to the public through the US National Technical Information Service.

In 1993 FEMA initiated GRAS reaffirmation (GRASr), a comprehensive re-evaluation of all FEMA GRAS flavour ingredients. As part of GRASr, the relevant scientific data which provide the basis for the Panel's conclusion of GRAS are presented for each group of structurally related flavour ingredients in a Group Summary, and for each individual flavour ingredient in an Interpretive Summary. While these summaries include only critical data relevant to the safety evaluation, it should be emphasized that the Panel has reviewed all available data. The Group Summaries and Interpretive Summaries supersede the SLRs.

II. Principles of GRAS assessment of flavour ingredients

The FEMA GRAS program, like every scientific program, has experienced periodic changes. However, its original underlying principles have been maintained and continue to be strengthened with time. Key elements that have been used historically by the Expert Panel and are fundamental to the GRAS assessment program include exposure, structural analogy, metabolism, pharmacokinetics and toxicology.

Exposure

Exposure data are critical to the safety assessment of flavour ingredients. Maximum exposure traditionally has been estimated using one of two principal methods. The possible average daily intake (PADI) is calculated by using the concentration of a flavour ingredient added to foods, and the amount of those foods consumed daily. A second method for estimating exposure is based on the volume of flavour ingredients that disappears from the marketplace (NAS,1970, 1975, 1982 and 1987; IOFI, 1995); that is, the annual volume of a substance reported to be sold by the industry for use as a flavour ingredient in food and beverages in the US. This estimate is

commonly recognized as the daily *per capita* intake. The accuracy of either method when used to estimate the intake of flavour ingredients depends on the validity of the input data and the assumptions inherent in the method of calculation.

PADI. The PADI is calculated using the concentration of a flavour ingredient added to foods within specific food categories, and the daily mean consumption of those food categories. It incorporates the assumption that *all* foods within a category (e.g. baked goods) are flavoured with the substance in question. This assumption, however, does not apply to most flavour ingredients which are used in only a few items within a category. For instance, methyl anthranilate is used to impart grape flavour in jelly donuts and pastries which account for only a small fraction of the baked goods consumed daily (e.g. bread, bagels, chocolate cake, etc.). The result is that the PADI is often a gross exaggeration of the potential exposure to individual flavour ingredients.

The annual production volume that would be required to account for the PADI often exceeds actual production volumes by two to four orders of magnitude. The exaggeration is most obvious for the vast number of flavour ingredients which have low annual volumes of use and appear in only a few brands of food in a particular food category. The PADI provides a better estimate of exposure for substances that have high (i.e. > 1,000,000 pounds) total annual production volumes and are used in the majority of foods within a category. Because of their unique organoleptic properties, the use of most flavouring substances is limited.

Daily *per capita* intake ("eaters only"). The daily *per capita* intake ("eaters only")* is determined from the disappearance of flavour ingredients from the marketplace (NAS,1970, 1975, 1982 and 1987; IOFI, 1995); that is, the annual total volume of a substance reported to be sold by the industry for use as a flavour ingredient in food and beverages. It is considered a conservative estimate because it incorporates the assumption that only 10% of the US population (i.e. the "eaters only") consumes the entire annual volume reported by the industry. An added margin of safety is provided by the assumption that only 60% of the actual US volume in use was reported in the annual industry survey. Results of repeated US industry surveys (NAS, 1970, 1975, 1982 and 1987) and a European industry survey (IOFI, private communication to FEMA, 1995) have revealed similar patterns of flavour use for both populations.

In comparison with the PADI, the daily *per capita* intake ("eaters only") has been shown to better approximate the actual intakes of flavour ingredients consumed by volunteers during a controlled study (Oser and Hall, 1977). The mean intakes from total observations (MITO) for 12 flavour ingredients in food were determined based on the consumption patterns of 12,000 volunteers over a 14-day eating

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*The *per capita* intake ("eaters only") is based on the assumptions that: (1) only 60% of the flavour volume was reported in the NAS survey and, therefore, the total annual volume was 1.67 times greater than reported; and (2) 10% of the population, the "eaters only", consumed that entire volume.

period (NAS/NRC, 1976). For the 12 substances in the study, the PADI was in the range from 10 to 100,000 times greater than the respective MITO estimates, whereas the *per capita* intake was 0.2 to 30 times the respective MITO. The *per capita* intake method is considered the preferred method by the FDA and the FEMA Expert Panel.

Factors affecting exposure to flavour ingredients. The unique properties of flavour ingredients, and the processes by which flavour ingredients are incorporated into the food supply must be considered when estimating exposure. Because of their organoleptic properties, most flavour ingredients are added to foods at low concentrations, generally less than 100 ppm. The concentration of flavour ingredients in foods as they are consumed is often much lower than the added concentration. Substantial loss occurs from food products prior to consumption through the evaporation of flavour ingredients, which are typically quite volatile. Loss associated with evaporation is significant for processed foods which are heated during processing. Similarly, flavour ingredients may be added to foods that are not ready-to-eat and therefore will be significantly diluted, during the food preparation or cooking process, prior to consumption. Although the concentration of flavour ingredients in food categories such as chewing gum or hard candy is typically an order of magnitude greater than in other foods, overall exposure remains low because these foods are consumed in relatively small quantities, and absorption of the flavour from the food is relatively low (e.g. 30–40% menthol absorbed from chewing gum).

Exposure to the flavour ingredient from its natural occurrence in food is considered by the Expert Panel. When quantitative data are available for naturally occurring flavouring substances, the Panel considers the Consumption Ratio as part of their evaluation. The Consumption Ratio describes the ratio between the quantity of a flavour ingredient consumed as a natural component of basic and traditional foods, and the quantity of the flavour ingredient consumed as a flavour added to foods when it is consumed by the same population over the same period (Stofberg and Kirschman, 1985). The majority of flavour ingredients with available quantitative data exhibit Consumption Ratios greater than 1, which indicates that exposure occurs predominantly as a result of its natural occurrence in traditional food. The ratio is an estimate of relative exposure and is not intended to justify the safety of a flavour ingredient. However, if the substance is recognized as safe based on its structure, metabolism, toxicology and use, its long-term consumption as a natural component of food supports and strengthens the conclusion of GRAS.

The role of structural analogy

The FEMA GRAS program is based on the philosophy that for substances for which the exposure is trivial, predictions regarding their safety can be reliably evaluated within the context of its structurally related group. For example, it is appropriate to consider data on menthol and other menthol derivatives when evaluating the safety of menthyl acetate, which is used at very low levels in a limited number of foods. The vast majority of flavour ingredients have trivial exposure and therefore can be evaluated using structural analogy. For substances with significant exposure, more extensive data on the individual substance may be required.

Toxicity is a function of chemical structure, and the magnitude of the toxic effect is a function of dose. Therefore, sound safety evaluation depends on understanding the toxic effects associated with structurally similar substances and the dose levels at which toxicity is observed. With few exceptions, structurally related flavour ingredients exhibit similar routes of metabolic detoxication, enzyme utilization and organ specific effects. In the absence of metabolic data on a specific substance, biotransformations usually can be predicted on the basis of the functional groups present, the metabolic options that these groups present and the known metabolism of structurally related compounds.

For the vast majority of flavour ingredients, no-observed-adverse-effect levels (NOAELs) from studies of structurally related substances are more than 100,000 times their exposure levels from use as flavour ingredients. Such large margins of safety would compensate for the slight variation in toxicity that may be associated with differences in structure (e.g. carbon chain length, substitution, stereochemistry, etc.).

Reaction equations are used in the Group Summaries and Interpretive Summaries to illustrate how important metabolic and biochemical transformations link the flavour ingredient to related substances or metabolites. Enzyme systems that catalyse these reactions are described to demonstrate the relationship between metabolism and endpoints of toxicity. In addition to other flavour ingredients, related substances may include fragrances, pheromones, pharmaceuticals and endogenous substances present in animals.

Metabolic fate

The potential target site for toxicity is a function of the chemical structure of the substance and its metabolites formed *in vivo*. Consequently, metabolic data have a critical role in the GRAS assessment process. Changes in metabolism should be consistent with changes in toxicity; a dose-dependent change from a detoxication to an intoxication metabolic pathway should correspond to a dose-related change in observed toxicity.

The availability of comprehensive metabolic and toxicologic profiles is limited. Most metabolism studies identify only major excretion metabolites in non-human animals, and do not account for 100% of

the dose. Despite these limitations, such metabolism studies are useful to GRAS assessment because their primary purpose is to identify the presence of available detoxication pathways. Additionally, metabolism studies often provide insight as to how structural variation among substances may be related to their differences in observed toxicity. For instance, the substantial difference in toxicity between the two structurally-related, alicyclic, terpenoid ketones, pulegone (p-menth-4(8)-en-3-one; Fig. A7) and carvone (p-menth-8-en-2-one; Fig. A5) can be understood when their different metabolic pathways are considered.

Specific enzyme systems utilized during metabolism of structurally related substances are described to characterize important cellular metabolites and determine concentration thresholds at which saturation of an enzyme may lead to secondary pathways of metabolism and different endpoints of toxicity. Species, sex, route of administration and dose potentially influence the pattern of metabolism and endpoints of toxicity. Therefore, the relevance of results obtained from metabolic studies in animals using high-dose levels must be critically evaluated before applying them to human safety.

Toxicology

In conjunction with their views on structural analogy, the Expert Panel believes that it is unwarranted to require toxicological studies for every flavour ingredient. The exposure to most flavour ingredients is very low, and their metabolic fate is either known or can be reasonably predicted. Therefore, subchronic or chronic studies available on a few members of a group of flavour ingredients may be sufficient for indicating the toxicological profile of all substances in the group. For individual substances that have significant exposures, particularly when metabolic fate is not well understood, toxicological studies may be required to support safety in use as a flavour ingredient.

Relevant toxicology studies of flavour ingredients typically include oral acute toxicity tests, and subchronic or chronic animal feeding studies. Chronic studies reporting adverse effects are evaluated in greater detail than similar studies in which minor or no effects were observed. The relationship between toxic potential and exposure is evaluated by comparing the estimated daily *per capita* intake ("eaters only")* to the NOAEL reported in an adequate subchronic or chronic feeding study for either the flavour ingredient or a structurally related substance.

While the Expert Panel recognizes that current study protocols are more rigorous and comprehensive than in the past, they believe that older studies which met the testing standards considered acceptable at the time of conduct may provide information that is still valuable today. Such studies are evaluated

on an individual basis to determine their relevance in the safety evaluation of flavour ingredients.

Mode of administration. For purposes of GRAS assessment, the most relevant mode of administration of flavour ingredients is that which mimics human exposure, ingestion in the diet. Some flavour ingredients are sensitive to the acidic and basic environments of the alimentary canal. Certain esters, lactones, sulfides, thiols and other substances may undergo spontaneous chemical changes prior to their absorption. Enzymes in the gastric juices, intestinal fluid and intestinal mucosa catalyse hydrolysis, and intestinal microflora provide a source for active reduction. Intravenous, inhalation and intraperitoneal routes of exposure preclude these important biochemical transformations which occur prior to absorption. Thus, data from studies using routes of administration other than dietary ingestion are given less weight in the GRAS assessment.

The means of ingestion is an important factor in determining the relevance of data. Repeated oral administration of a substance by corn oil gavage indeed places the substance in the alimentary system, but resulting peak plasma levels are significantly higher than those following dietary inclusion. Consequently, the physical effects of repeated gavage and excessive corn oil intake subject the test species to unnatural conditions which may result in toxicity unrelated to the flavour ingredient (Larson et al., 1993). The high mortality observed in lifetime gavage studies (NTP, 1992a) attests to this inferior protocol. The results of studies in which flavour ingredients are ingested as part of a normal diet are most relevant for safety assessment.

In the absence of adequate oral studies, results of inhalation studies may be used for low molecular weight, volatile substances that exhibit similar pathways of metabolism and excretion regardless of the mode of administration. As a crude approximation, NOAELs in inhalation studies are converted into equivalent-absorbed concentrations in order to estimate a safety factor. The Expert Panel emphasizes that these estimates are based on many assumptions that may not be accurate and should only be considered when exposure from use as a flavour ingredient is many orders of magnitude less than exposure from inhalation.

Relevance of other toxicology studies. In addition to subchronic and chronic toxicity studies, other available studies may be considered by the Expert Panel during the GRAS assessment if determined to be relevant. For example, genotoxicity tests have been performed for a wide variety of flavour ingredients. Under limited circumstances, these tests are useful for interpreting adverse effects in chronic feeding or carcinogenicity studies, if produced in well-validated test systems. For instance, the results of mutagenicity and genotoxicity tests were used to interpret the results of a chronic study of isophorone (Bucher et al., 1986) (see Section III.A.2.a.).

Genotoxicity tests are not required by the Expert Panel but may be considered to evaluate chronic or carcinogenicity studies, if deemed appropriate. In general, the effects observed for high concentrations of a substance suspended in an artificial cellular environment have limited relevance to the human consumption of low concentrations of flavour ingredients which are metabolized *in vivo* by efficient detoxication mechanisms.

Other available studies that evaluate toxicological endpoints such as reproductive toxicity are considered in the GRAS assessment of flavour ingredients where available. The interpretation of these studies takes into consideration factors such as dose–response information, the relationship of the minimum effective dose to the typically low exposure to flavour ingredients, the relevance of findings at maternally toxic dose levels to human exposure levels, and pharmacokinetic and metabolic differences between experimental animals and humans.

PART 2—ALICYCLIC SUBSTANCES USED AS FLAVOUR INGREDIENTS

I. Introduction

Chemical identity

This review summarizes the key data relevant to the safety evaluation of 119 alicyclic substances used as flavour ingredients. The review is organized into three sections based on functional group: Section II contains 25 primary alcohols, aldehydes, carboxylic acids and related esters, and is subdivided into unsubstituted monocyclic (six) and substituted monocyclic and bicyclic (19) substances. Section III contains 81 ketones, secondary alcohols and related esters and is subdivided according to the location of the functional group: on the ring (60) or on the alkyl substituent attached to the ring (21) such as β-ionone. The substances with the functional group on the ring are subdivided into unsubstituted monocyclics (five), substituted monocyclics (34) and bicyclic and macrocyclic (21) substances including camphor and nootkatone derivatives. The substituted monocyclics consist of the cyclopentanone and cyclohexanone derivatives (10), and three groups of monocyclic terpenes† with specific functions as flavour ingredients: carvyl (nine), menthyl (10) and pulegone (five) derivatives. Section IV contains 13 tertiary alcohols and related ketones and esters, most of which are derived from α-terpineol.

Exposure

A large number (119) of alicyclic substances are used as flavour ingredients, but a significant fraction of the intake is associated with parent substances (e.g. menthol and menthone) of each particular subgroup

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†A terpene is a substance formed by the mevalinic acid pathway in plants and animals. Substances formed by this pathway are composed of two or more five-carbon isoprene (i.e. 2-methylbutyl) units.

(e.g. menthyl). Other structurally related substances (e.g. menthyl lactate) in a subgroup generally provide similar flavour characteristics but have specialized use in selected products of a food category. The annual volume of use of these latter substances as flavour ingredients is usually relatively insignificant (< 100 kg/year).

Of the alicyclic substances reviewed, only 12 have volumes greater than 500 kg/year (see Tables B1–B19). Of the 12, only seven have volumes greater than 1000 kg/year, three have volumes greater than 10,000 kg/year, and two have volumes greater than 50,000 kg/year (NAS, 1987). The majority of the high volume substances are parent alcohols (menthol) or ketones (carvone) of a subgroup of flavour ingredients with a specific flavour use (e.g. peppermint and spearmint).

Alicyclic flavour ingredients with a specialized function ("cool mint" of menthol or spearmint flavour of carvone) tend to be used at higher levels. For example, average maximum use levels reach 2500 ppm for menthol in soft candies and 350 ppm for carvone in spearmint flavoured chewing gum. Substances associated with more general flavour characteristics such as citrus are normally used at lower levels (< 100 ppm for α-terpineol), and in a wider range of food categories (e.g. baked goods, frostings, and soft candy). The majority of substances with low volumes (< 100 kg/year) are used in fewer food categories usually at levels lower than 20 ppm.

As the majority of alicyclic substances used as flavour ingredients are mono- and bicyclic terpenes synthesized by plants, it is not surprising that they have been found to occur naturally in a wide variety of foods. It has been estimated that the intake levels of menthol, menthone and carvone as naturally occurring components of food are two to five times their respective intake levels from use as flavour ingredients (Stofberg and Grundschober, 1987; Stofberg and Kirschman, 1985). The vast majority of other alicyclic substances for which quantitative data are available are consumed as naturally occurring components of food at levels more than 10 times their intake from use as flavour ingredients (see Appendix B).

Metabolism

The metabolic options available to alicyclic substances increase with an increase in the number and types of functional groups and ring substituents in the molecule. If a primary alcohol, aldehyde or carboxylic acid function is present on an alkyl side-chain, the substance may undergo β-oxidation and cleavage. If the number of carbons in the side-chain is even, β-oxidation may lead to cleavage of the alicyclic ring (Voet and Voet, 1990). If the number is odd, formation of a cycloalkane carboxylic acid may result. In the latter case when cyclohexane-carboxylic acid is produced, the acid may undergo partial ring dehydrogenation or aromatization prior to excretion (Brewster, 1997a,b).

Alicyclic terpenoid primary alcohols (e.g. perilla alcohol) which contain alkyl ring substituents generally oxidize to the corresponding carboxylic acid, conjugate with glucuronic acid, and are excreted. Terpenoid aldehydes (e.g. perillaldehyde) also undergo oxidation to the corresponding carboxylic acid or, to a lesser extent, reduction to the corresponding alcohol with subsequent conjugation and excretion (Haag and Gould, 1994; Ishida, 1989). If the substance has an endocyclic alkene function and is excreted into the bile, intestinal microflora may promote hydrogenation of the double bond. Excretion metabolites, therefore, may include conjugates of the reduced form of the alcohol or acid (Ishida, 1989).

As with acyclic substances, simple, unsubstituted, alicyclic secondary alcohols and ketones (e.g. cyclopentanol and cyclopentanone) are readily interconverted by oxidation–reduction reactions. For low molecular weight, polar alicyclic substances (e.g. cyclopentanone) the ketone is stereoselectively reduced by cytosolic carbonyl reductases to yield the secondary alcohol which is conjugated primarily with glucuronic acid (James and Waring, 1971). The resulting conjugate may pass into the bile and be excreted in the faeces or, more importantly, enter enterohepatic circulation and be excreted in the urine (Yamaguchi et al., 1994). For higher molecular weight, more lipophilic substances or those with sterically hindered functional groups, oxidation of a ring position by non-specific cytochrome P-450 mixed-function oxidases may compete with reduction of the ketone function or oxidation of the alcohol function (Asakawa et al., 1986; Hildebrandt, 1902). If the alicyclic alcohol or ketone contains an endocyclic double bond, oxidation or hydrogenation of the alkene may lead to additional metabolites.

If a secondary alcohol or ketone function is located on a ring containing alkyl substituents, as in simple terpenoid derivatives, oxidation of the alkyl substituents competes with oxidation–reduction reactions of the alcohol or ketone function. If the substance contains allylic or tertiary hydrogens, the rate of oxidation increases often leading to polyoxygenated metabolites (Dutertre-Catella et al., 1978). The number of possible polyoxygenated metabolites increases with an increase in the types of alkyl ring substituents (e.g. methyl and isopropyl substituents) and the presence of an alkene (endocyclic and/or exocyclic) in which hydrogenation may precede or succeed oxidation of the substituent (Madyastha and Raj, 1993; Nelson et al., 1992; Yamaguchi et al., 1994). As molecular weight and molecular complexity increases, as in di- (e.g. nootkatone) and tri-terpenoid (e.g. abeitic acid) derivatives, oxidation of alkyl substituents becomes a more important metabolic pathway. Substances exhibiting greater lipophilicity may undergo oxidation of the secondary alcohol function to the corresponding ketone in addition to oxidation of alkyl substituents (Asakawa et al., 1986).

If the functional group is on an alkyl side-chain, as in the ionone derivatives, the ketone may be reduced to the corresponding alcohol. In addition, oxidation of activated ring positions may also occur (Ide and Toki, 1970; Kraft et al., 1991; Vane et al., 1990).

Tertiary alcohol functions are relatively stable in vivo and eventually are excreted as the glucuronic acid conjugates. Ring alkyl substituents of tertiary alcohols are generally oxidized to diols and hydroxyacids, similar to that of secondary alcohols and ketones. Tertiary alcohols with ring unsaturation would yield products of hydrogenation or oxidation of the alkene (Horning, 1976; Ventura et al., 1985).

Toxicology

With the exception of pulegone, alicyclic substances exhibit very low oral acute toxicity (i.e. LD_{50} > 1000 mg/kg). Rodent LD_{50} values in the range from 1000 to more than 5000 mg/kg have been reported for 83 of the 119 alicyclic substances. The majority of these LD_{50} values are greater than 2000 mg/kg. A rat oral LD_{50} value of 470 mg/kg has been reported for pulegone (Moreno, 1975).

Oral subchronic studies have been reported for at least one member of each group reviewed in this summary except the iso-pulegone derivatives and the unsubstituted monocyclics. Repeated dose studies are available for the parent alcohol, ketone or acetate ester in each group, such as terpinyl acetate, ionone (α and β) and perilla alcohol. The parent substances generally account for the majority of use as flavour ingredients. For example, subchronic and chronic studies exist for carvone which accounts for more than 96% of the annual volume of all carvyl derivatives use as flavour ingredients (NAS, 1987). More than one subchronic study usually exists for groups containing substances with considerable structural variation (i.e. cyclohexyl and cyclopentyl ketones, sterically hindered ketones and α,β-unsaturated ketones; see Section III. A. 2.).

In most of the reported subchronic studies, no adverse effects were observed at any dose level. In studies that showed adverse effects (e.g. studies for α- and β-ionone and iso-bornyl acetate), NOAELs were in the range from 15 mg/kg/day to 500 mg/kg/day. The dose levels that resulted in no adverse effects for a parent or representative substance was at least 1000 times the total daily per capita intake of all subgroup members as flavour ingredients. 2-Year oral carcinogenicity bioassays have been performed for menthol and carvone which have the highest reported annual volumes (54,400 kg for menthol and 52,300 kg for carvone) of use of all alicyclic substances used as flavour ingredients (NAS, 1987). No evidence of carcinogenicity or toxicity was reported in either study at dose levels (600 mg/kg/day for d,l-menthol or 750 mg/kg/day for d-carvone) greater than 1000 times the daily per capita intake ("eaters only")* of 0.17 mg/kg from use of carvone or menthol as flavour ingredients.

II. Primary alcohols, aldehydes, carboxylic acids and related esters

A. Monocyclic, unsubstituted

Chemical identity and exposure. Group II. A. contains six unsubstituted monocyclic substances used as flavour ingredients including cyclohexanecarboxylic acid, cyclohexaneacetic acid, and related esters (see Fig. A1). They are used as flavour ingredients up to average maximum levels of 25 ppm (see Table B1). In the majority of food categories, usual levels of use are below 10 ppm.

The reported annual volumes of unsubstituted monocyclics used as flavour ingredients are less than 50 kg/year (NAS, 1987) (see Table B1). On the basis of the total annual volumes reported for all six unsubstituted monocyclics in the most recent survey (NAS,1987), the estimated total daily *per capita* intake ("eaters only")* of unsubstituted monocyclics from use as flavour ingredients is 0.1 μg/kg.

Three substances in this group have been detected as natural components of foods: cyclohexanecarboxylic acid (beef extract) and the corresponding methyl (vanilla) and ethyl (rum) esters (CIVO–TNO, 1989).

Metabolism. Esters of cyclohexanecarboxylic acid, cyclohexaneacetic acid and higher homologues hydrolyse to the component alcohol and carboxylic acid (Ford and Moran, 1978). The component acids may undergo β-oxidation and cleavage of the side-chain. If the side-chain contains an odd number of carbons, the resulting metabolite is cyclic; if it contains an even number of carbons, ring cleavage occurs to yield acyclic metabolites (see Fig. 1). The component acid of ethyl cyclohexanepropionate undergoes oxidation to its homologue cyclohexanecarboxylic acid which is subsequently aromatized to benzoic acid and excreted mainly as the hippuric acid.

In perfused rat liver, cyclohexanecarboxylic acid is primarily metabolized to hippuric acid. Small amounts of cyclohexylcarbonyl glucuronide, hexahydrohippuric acid, 3,4,5,6-tetrahydrohippuric acid, unchanged cyclohexanecarboxylic acid and benzoic acid were also detected (Brewster *et al.*, 1977a). Benzoyl glucuronide was found in the bile of rats as an *in vivo* metabolite of cyclohexanecarboxylic acid (Brewster *et al.*, 1977b). Cyclohexylcarbonyl glucuronide becomes the predominant metabolite as the amount of cyclohexanecarboxylic acid increases and the hippuric acid pathway becomes saturated. Benzoic acid has been identified as a urinary metabolite following oral administration of cyclohexanecarboxylic acid in humans, dogs and rabbits (Bernhard and Caflisch-Weill, 1945). Substances with an even number of carbons do not undergo the penultimate step of aromatization to benzoic acid, but continue to undergo β-oxidation to yield ring cleavage products, primarily dicarboxylic acids which presumably enter the citric acid cycle. In the case of cyclohexaneacetic acid, an increased excretion of succinic acid has been observed (Williams, 1959).

Toxicology. Oral LD_{50} values greater than 2000 mg/kg have been reported for four of the six unsubstituted monocyclics and demonstrate very low oral acute toxicity (see Table B2).

Although no subchronic toxicity studies have been reported for this group of unsubstituted monocyclics, a study has been reported for a structurally related monocyclic ester that was recently reviewed by the FEMA Expert Panel as part of a group of allyl esters. Allyl cyclohexanepropionate (FEMA No. 2026; see Fig. A1) is expected to be hydrolysed *in vivo* to form allyl alcohol and cyclohexanepropionic acid. The latter would undergo β-oxidation to form cyclohexanecarboxylic acid (Williams, 1959).
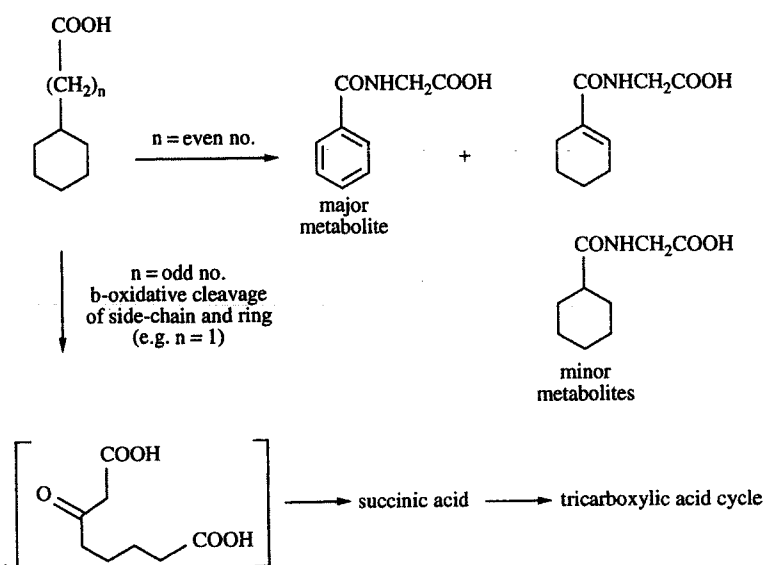

Fig. 1. Recognized metabolism of cyclohexanecarboxylic acid derivatives in animals.
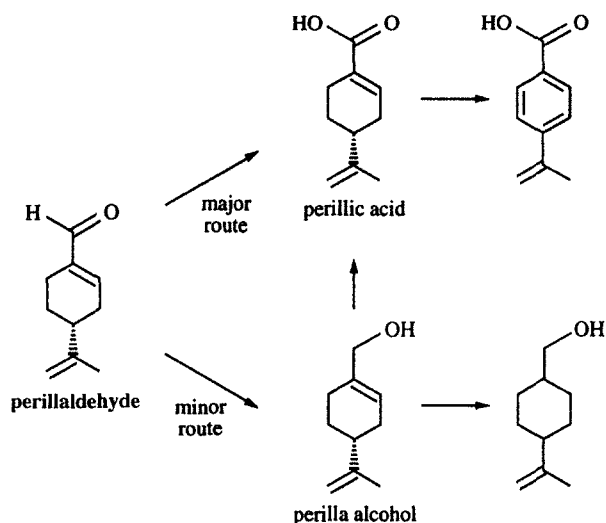
Fig. 2. Recognized metabolism of perillaldehyde in animals.

No adverse effects were reported when allyl cyclohexanepropionate was added to the diet of rats providing dose levels of 50 or 125 mg/kg daily for 27–28 weeks (Bar and Griepentrog, 1967) or 1 year (Hagan et al., 1967), respectively. No adverse effects were observed when rats were administered 2, 20 or 100 mg allyl cyclohexanepropionate/kg in sesame oil by stomach tube, 5 days/week for 3 months (Shelanski, 1953). No adverse effects were reported when rats were maintained on diets providing 0.1% and 0.25% allyl cyclohexanepropionate for 13 weeks (Paynter, 1957). The 0.25% dose level that produced no adverse effects in rats is calculated (FDA, 1993) to result in an average daily intake of 125 mg allyl cyclohexanepropionate/kg and is more than 1,000,000 times the total daily per capita intake ("eaters only")* of 0.1 µg/kg of the six unsubstituted monocyclics used as flavour ingredients. The large margin of safety would accommodate any anticipated difference in toxicity between these unsubstituted monocyclics and the structurally related unsubstituted monocyclic, allyl cyclohexanepropionate.

B. Monocyclic and polycyclic, substituted

Chemical identity and exposure. Group II. B. reviewed here is comprised of 19 substituted monocyclic and polycyclic substances (see Fig. A2). They are used as flavour ingredients up to average maximum levels of 35 ppm, except for tetrahydrocuminic acid, which is used up to 200 ppm (see Table B3). As reported in the most recent survey (NAS, 1987), no substance in this group has a reported annual volume of use as a flavour ingredient greater than 15 kg/year, except for 2-formyl-6,6-dimethylbicyclo(3.1.1)heptene (myrtenal), which has an annual volume of 150 kg/year (see Table B3). On the basis of the total annual volume reported in the most recent survey (NAS, 1987) for all 19 substituted monocyclics and polycyclics used as flavour ingredients, the

estimated total daily per capita intake ("eaters only")* is 0.6 µg/kg. More 80% of the intake is derived from consumption of myrtenal as a flavour ingredient.

16 of the 19 substances have been reported to occur naturally in food (CIVO–TNO, 1989). They have been identified mainly in a wide variety of citrus fruits, berries and related fermented products. Concentrations in citrus fruit peel oils approach 5000 ppm for perilla alcohol, 2000 ppm for perillaldehyde and 50 ppm of myrtenal. Three of the four bicyclic terpenes derived from myrtenol are components of peppermint and spearmint, while the tricyclic terpene santalol and the corresponding acetate ester are found in artemisia and sandalwood oils.

Metabolism. In animals, monocyclic terpenoid primary alcohols (e.g. perilla alcohol) and monocyclic (e.g. perillaldehyde), bicyclic (e.g. myrtenal) and aromatic (e.g. cuminaldehyde) aldehydes are oxidized primarily to the corresponding acid. In a minor pathway, the aldehyde may be reduced to the alcohol and excreted as the glucuronide. If an endocyclic double bond is present, the metabolite may be reduced, presumably by gut microflora. The acid metabolite may undergo aromatization of the ring to yield a hippuric acid derivative which may be excreted as the glycine conjugate (see Fig. 2).

In rats, perilla alcohol (i.e. p-mentha-1,8-dien-7-ol) is oxidized mainly to perillic acid which is the major plasma metabolite. To a small extent, the endocyclic alkene is hydrogenated to yield dihydroperillic acid (Haag and Gould, 1994). In rabbits, perillaldehyde is oxidized mainly to p-menth-1,8-dien-7-carboxylic acid (i.e. perillic acid). Perillic acid is converted in part to p-isopropylbenzoic acid by aromatization of the cyclohexene ring and reduction of the isopropenyl double bond. To a lesser extent, perillaldehyde is reduced to perillyl alcohol which is selectively

hydrogenated to yield *p*-menth-8-en-7-ol (see Fig. 2). Analogous oxidation and reduction metabolites have been reported in rabbits given the bicyclic aldehyde myrtenal and aromatic aldehyde cuminaldehyde (Ishida *et al.*, 1989).

In rabbits, an oral dose of myrtenal is metabolized mainly to the corresponding acid which is excreted presumably as the glucuronic acid conjugate. Cleavage of the bicyclic ring to yield perillic acid has also been observed. To a lesser extent, the aldehyde is either reduced to myrtenol which is conjugated with glucuronic acid and excreted, or undergoes hydrogenation of the double bond probably by reduction by intestinal microflora (Ishida *et al.*, 1989) (see Fig. 3). Humans exposed to sawmill dust containing myrtenol excreted the glucuronic acid conjugate of myrtenol in the urine (Eriksson and Levin, 1990). Reduction of the aldehyde to the corresponding alcohol may be more important in the metabolism of sterically hindered aldehydes.

If the cyclic terpene contains an alkyl substituent of greater chain length than C_3, the primary alcohol, aldehyde, acid or related ester may undergo β-oxidation and cleavage of the side-chain to yield lower molecular weight carboxylic acid metabolites. On the basis of elemental analysis of the urinary metabolites of rabbits given repeated oral doses of santalol (i.e. a tricyclic primary alcohol containing a C_6 alkyl substituent), a metabolite was formed by loss of an isoprene (C_5) unit from santalol (Hildebrandt, 1902). Structurally related triterpenoid alcohols (farnesol) and ketones (geranylgeranylacetone) undergo β-oxidation and cleavage to produce chain-shortened polar diacid fragments. For example, C-7, C-9 and C-11 diacid fragments have been identified in the urine of rats given an oral dose of the geranylgeranylacetone (Nishizawa *et al.*, 1987).

Toxicology. 14 of the 19 unsubstituted monocyclics and polycyclics have reported oral LD_{50} values, and all exhibit very low oral acute toxicity. Rodent oral LD_{50} values are in the range from 1000 ml/kg to 5700 mg/kg (see Table B4).

A subchronic study (BIBRA, 1976) and a limited study (Haag and Gould, 1994) have been reported for two aldehydes in this group and resulted in no observed adverse effects. (2,2,3-Trimethylcyclo-pent-3-en-1-yl)acetaldehyde (i.e. campholenic aldehyde) was administered in corn oil by gavage to rats at levels providing a daily dose of 11.9 mg/kg for 90 days. Increased weight gain and thickening of the alveoli of males were the only effects observed and were not attributed to the test substance (BIBRA, 1976).

In a limited study designed to evaluate the antitumorigenic effect of perilla alcohol and its metabolites, groups of rats were given a single dose of 50 mg dimethylbenz[*a*]anthracene/kg. On palpation of the first mammary tumour, animals were placed on diets containing 0, 0.5%, 1.0%, 1.5% or 2% perilla alcohol for 15 weeks. Maximum daily intake of perilla alcohol was calculated to be 0, 410, 880, 1280 or 1610 mg/kg, respectively. A significant dose-related decrease in tumour growth (25–75%) was observed. No observable signs of toxicity were reported at any dose level. Body weight gain was slightly depressed at the 1610 mg/kg/day intake level (Haag and Gould, 1994).

The dose levels of 11.9 and 50 mg/kg/day that resulted in no adverse effects are more than 10,000 times the estimated total daily *per capita* intake ("eaters only")* of 0.6 μg/kg of the 19 substituted bicyclic and polycyclic substances from use as flavour ingredients.

III. Ketones, secondary alcohols and related esters

A. Functional group on the ring:
1. Unsubstituted monocyclics

Chemical identity and exposure. Group III. A. 1. comprises five unsubstituted monocyclic esters
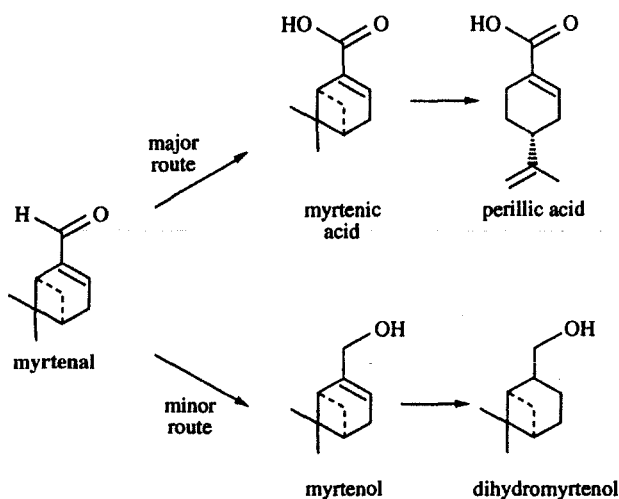

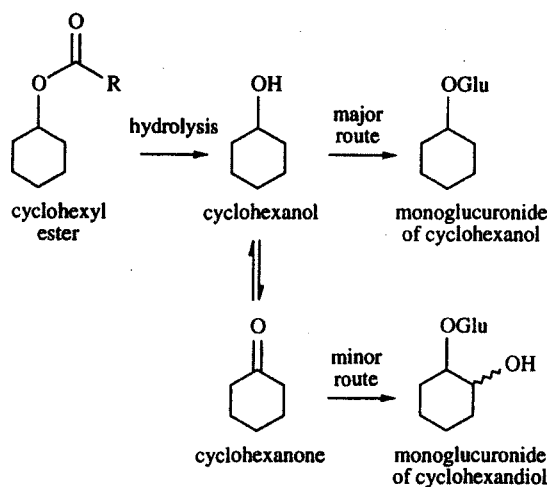Fig. 3. Recognized metabolism of myrtenal in rabbits.

Fig. 4. Proposed metabolic fate of cyclohexyl esters in humans.

derived from cyclohexanol and simple aliphatic carboxylic acids C_1 to C_5 (see Fig. A3). They are usually used as flavour ingredients at levels below 10 ppm, but can be used up to an average maximum level of 105 ppm (see Table B5). With one exception, these substances were reported in the most recent survey to have annual volumes of use as flavour ingredients of less than 5 kg (NAS, 1987). The annual volume reported for cyclohexyl acetate from use as a flavour ingredient is 255 kg (NAS, 1987). On the basis of the annual volumes reported in the most recent survey (NAS, 1987), the estimated total daily per capita intake ("eaters only")* is 0.8 μg/kg of all five unsubstituted monocyclics used as flavour ingredients. Cyclohexyl acetate accounts for more than 99% of the total intake.

Cyclohexyl acetate and cyclohexyl butyrate have been reported to occur naturally in foods including soybean, sauerkraut and kumquat (CIVO–TNO, 1989).

Metabolism. The unsubstituted monocyclic esters are expected to rapidly hydrolyse to cyclohexanol and the component aliphatic carboxylic acids by classes of enzymes recognized as carboxylesterases (Ford and Moran, 1978; Heymann, 1980; White et al., 1990), the most important of which are the B-esterases. In mammals, these enzymes occur in most tissues throughout the body (Anders, 1989; Heymann, 1980) but predominate in the hepatocytes (Heymann, 1980).

The metabolic fate of cyclohexanol is similar to that of simple acyclic aliphatic secondary alcohols (see Fig. 4). The glucuronic acid conjugate of cyclohexanol and cyclohexanone was found in the urine of workers occupationally exposed to a mixture of hexanes including cyclohexane (Governa et al., 1987; Perbellini et al., 1981). Cyclohexanol forms conjugates of glucuronic acid in rabbits (Sasaki, 1917; Treon et al., 1943) and dogs (Pohl, 1925) and sulfate

in rabbits (Sasaki, 1917; Treon et al., 1943) prior to excretion in the urine. In addition to mainly unchanged cyclohexanol and small amounts of the glucuronic acid conjugate, rabbits have also been reported to oxidize small amounts of cyclohexanol to the cis- and trans-1,2-cyclohexanediol, which are excreted primarily as monoglucuronide conjugates (Elliott et al., 1943). The monoglucuronide conjugate of the diol has been detected in the urine of rats given cyclohexanone oxime (Parmer and Burka, 1991). Presumably, the diol forms by hydroxylation and subsequent reduction of cyclohexanone. In rats and rabbits, cyclohexanone or cyclopentanone are reduced to the corresponding secondary alcohol which is excreted as the glucuronic acid conjugate (Elliott et al., 1943; James and Waring, 1971). Rabbits given large doses of cyclohexanol also excreted trace amounts of hydroxycyclohexyl mercapturic acid and cis-2-hydroxycyclohexylmercapturic acid in the urine (Elliott et al., 1943). In rats and rabbits, the structurally related substances 2-, 3- and 4-methylcyclohexanone are reduced to the corresponding secondary alcohols and excreted in the urine as the glucuronic acid and sulfate conjugates (Elliott et al., 1943; Tao and Elliott, 1962; Treon et al., 1943).

Toxicology. Unsubstituted monocyclic esters exhibit very low oral acute toxicity as demonstrated by rodent oral LD_{50} values greater than 5000 mg/kg reported for cyclohexyl acetate and cyclohexyl butyrate and 6600 mg/kg for cyclohexyl acetate. The component alcohol cyclohexanol has oral LD_{50} values in the range from 2060 mg/kg in rats to 2600 mg/kg in rabbits (see Table B6).

The subchronic toxicity of the unsubstituted monocyclics is supported by studies on the component alcohol cyclohexanol and a structurally related alcohol methylcyclohexanol (see Fig. A3). Because cyclohexanol has widespread use as an industrial solvent, most of the studies have been limited to the inhalation or intraperitoneal routes of administration. The toxicity of cyclohexanol is expected to be greater, particularly when administered by inhalation or intraperitoneally, than its esters which are ingested as flavour ingredients at very low levels.

Rabbits were exposed to atmospheres containing either 145 ppm (0.58 mg/litre) or 272 ppm (1.09 mg/litre) cyclohexanol for 6 hours/day, 5 days/week for 10 weeks. Degenerative changes of the liver, myocardium and kidney were observed only at the highest dose level. Liver and kidney effects at the lowest dose level were described as slight degenerative changes. Rabbits were exposed to atmospheres containing 121 ppm and 232 ppm methylcyclohexanol (i.e. a mixture of 2-, 3- and 4-methylcyclohexanol) for the same duration (Treon et al., 1943). As methylcyclohexanol is absorbed rapidly regardless of the mode of administration (Treon et al., 1943), it is reasonable to extrapolate inhalation exposure levels to probable equivalent systemic levels. On the basis

of the conservative assumption that only 50% of inhaled methylcyclohexanol is absorbed by the lungs, the 121 ppm concentration is calculated (Beliles and Schulz, 1993) to provide an absorbed dose of approximately 45 mg/kg.

No adverse effects were reported in workers exposed to atmospheres containing up to 0.456 mg cyclohexane/litre which was principally metabolized to cyclohexanol and excreted in the urine as the glucuronic acid conjugate (Perbellini *et al.*, 1981).

A limited study was conducted to evaluate the neurotoxicity of cyclohexanol and cyclohexanone in rats. Cyclohexanol was administered in two intraperitoneal injections/day at 200 mg/kg doses (i.e. 400 mg/kg/day), 5 days/week for up to 6 weeks. Cyclohexanone was administered at 400 mg/kg/day for up to 13 weeks. No evidence of neurotoxicity was reported for either substance (Perbellini *et al.*, 1981).

The levels of 45 mg methylcyclohexanol/kg/day or 400 mg cyclohexanol/kg/day that produced no adverse effects are more than 10,000 times the total daily *per capita* intake ("eaters only")* of 0.8 µg/kg of all five unsubstituted monocyclics used as flavour ingredients. The large margin of safety would accommodate any anticipated difference in toxicity between the cyclohexyl esters and the component alcohol cyclohexanol or a structurally related alcohol.

2. Substituted monocyclics

There are four subgroups of substituted monocyclic substances used as flavour ingredients. Subgroup (a) cyclopentanone and cyclohexanone derivatives includes terpenoid and non-terpenoid substances which have no common functional use as flavour ingredients. Subgroups (b) carvone derivatives, (c) menthone derivatives and (d) pulegone derivatives are terpenoids that do serve a specific function as flavour ingredients. For example, menthone derivatives exhibit a "cool mint" character. The substances in subgroups (b), (c) and (d) all have an oxygenated functional group on the ring and a terpene (1-methyl-4-isopropylcyclohexane) nucleus. They are either saturated (menthol) or unsaturated containing exocyclic (pulegone) and/or endocyclic (carvone/piperitone) double bonds.

a. Cyclopentanone and cyclohexanone derivatives: chemical identity and exposure. Group III. A. 2. a. contains 10 substituted monocyclic ketones that are derivatives of cyclopentanone and cyclohexanone (see Fig. A4). This group includes terpenoid and non-terpenoid substances which have no common functional use as flavour ingredients. The cyclopentanone and cyclohexanone derivatives are used as flavour ingredients up to average maximum levels of 30 ppm, except for 2-*sec*-butylcyclohexanone which is normally used up to 150 ppm, and up to 1100 ppm in chewing gum (see Table B7). In the majority of food categories, these flavour ingredients are usually used below 10 ppm. On the basis of the most recent

survey (NAS, 1987), the reported annual volumes for the cyclopentanone and cyclohexanone derivatives are below 10 kg except for 3-methyl-2-(2-pentenyl)-2-cyclopenten-1-one which has a volume of 50 kg/year (see Table B7). On the basis of the reported annual volumes (NAS, 1987), the estimated total daily *per capita* intake ("eaters only")* of the 10 substances in this group from use as flavour ingredients is approximately 0.3 µg/kg.

Five cyclopentanone and cyclohexanone derivatives have been reported to occur naturally in food as components of coffee, tea, beer, rice, nuts and berries (CIVO–TNO, 1989).

Metabolism. In general, alicyclic ketones may be reduced to the corresponding secondary alcohol and excreted primarily as the glucuronic acid conjugate. If a double bond is present, it may be reduced to the corresponding dihydro derivative (Krasavage *et al.*, 1982). For metabolites excreted into the bile, reduction of the double bond would most likely be associated with gut microflora. Endocyclic double bonds are more prone to reduction compared with exocyclic double bonds. In addition to reductive pathways, alicyclic ketones containing an alkyl side-chain may undergo oxidation of the side-chain to form polar metabolites which are excreted as the glucuronide or sulfate conjugates in the urine, and to a lesser extent, in the faeces.

The metabolic fate of cyclopentanone and cyclohexanone derivatives, such as isophorone, has been studied in mammals. In rabbits, isophorone is metabolized to 5,5-dimethyl-1-cyclohexen-3-one-1-carboxylic acid by way of allylic oxidation of the β-methyl group (Dutertre-Catella *et al.*, 1978; Truhaut *et al.*, 1970). Reduction of the ketone function and hydrogenation of the alkene function to yield isophorol and dihydroisophorone, respectively, were also observed (Dutertre-Catella *et al.*, 1978) (see Fig. 5). Analogous modes of metabolic detoxication have been reported for other monocyclic ketones (Ishida *et al.*, 1989; Williams, 1940). Based on an *in*

Table 1. Incidence of renal neoplasms associated with administration of isophorone to rats by gavage for 103 weeks

		No. of neoplasms/no. of animals given isophorone (mg/kg)	
	Control	250	500
Males			
Nephropathy	49/50	47/50	46/50
Tubule mineralization	1/50	31/50	20/50
Renal tubule hyperplasia	0/50	1/50	4/50
Renal tubule adenoma[a]	0/50	0/50	2/50
Renal tubule adenocarcinoma[a]	0/50	3/50	1/50
Females			
Nephropathy	21/50	39/50	32/50
Tubule mineralization	10/50	4/50	2/50
Renal tubule hyperplasia	0/50	0/50	1/50
Renal tubule adenoma[a]	0/50	0/50	0/50
Renal tubule adenocarcinoma[a]	0/50	0/50	0/50

[a]Historical incidence of tubular cell adenoma or adenocarcinoma: 4/1091 (0.4%).

vitro study, glutathione conjugation at the β-carbon may also occur as a minor detoxication pathway (Portoghese *et al.*, 1989). Similar pathways of metabolic detoxication are expected in humans.

Toxicology. The very low oral acute toxicity of cyclopentanone and cyclohexanone derivatives is demonstrated by rodent oral LD_{50} values in the range from 1870 to more than 5000 mg/kg which have been reported for five of the 10 substances in this group (see Table B8).

The subchronic toxicity of the cyclopentanone and cyclohexanone derivatives is supported by 13-week oral studies in rats which have been reported for four ketones in this group: the sterically hindered cyclohexanones, 2-*sec*-butylcyclohexanone and tetra-methylethylcyclohexenone; an α,β-unsaturated cyclopentanone, 2-hexylidene-cyclopentanone; and an α,β-unsaturated cyclohexenone, isophorone.

When rats were provided with 2-*sec*-butylcyclohexanone in food for 13 weeks at levels estimated to result in an average daily intake of 0, 160, 370 or 900 mg/kg/day, no adverse effects were observed at the two lowest dose levels. Decreased weight gain, particularly in female rats, and increased mortality were reported at the 900 mg/kg/day dose level (Hummler, 1969).

Decreased food consumption, decreased food efficiency, decreased weight gain and increased liver and kidney weights were reported when tetra-methylethylcyclohexenone in gum arabic solution was added to the diet of rats at a concentration of 6000 ppm for 13 weeks. Cellular desquamation of the renal, distal convoluted tubule was also noted in both male and female rats. Evidence of chronic testicular lesions and atrophic seminal vesicles was reported in test males (X. Firmenich and X. Cie, private communication, 1963). The dose level is calculated (FDA, 1993) to be 300 mg/kg/day.

In a multiple-dose follow-up study, rats were maintained on diets containing tetramethylethylcyclohexenone in gum arabic at concentrations of 336, 560 and 672 ppm for weeks 0–4, 5–10 and 11–13, respectively (Posternak *et al.*, 1969). Resulting average intake levels were calculated to be 40.51 mg/kg/day for males and 47.62 mg/kg/day for females. No behavioural or physical changes were observed. On the basis of the results of haematological and histological examinations, no significant differences were reported between test and control animals.

The α,β-unsaturated cyclopentanone 2-hexylidene-cyclopentanone was added to the diet of rats in an aqueous solution of arabic gum for 13 weeks. Concentrations in the food were 26 ppm for the first 4 weeks of the study, 43 ppm for weeks 5–10 and 52 ppm for weeks 11–13. No adverse effects were reported at calculated average daily intake levels of 2-hexylidenecyclopentanone of 2.90 mg/kg for males and 3.37 mg/kg for females (Posternak *et al.*, 1969).

The α,β-unsaturated cyclohexenone, isophorone, was administered to rats and mice 5 days/week by

Table 2. Incidences of hepatocellular neoplasms associated with administration of isophorone to mice by gavage for 103 weeks

	No. of neoplasms/no. of animals given isophorone (mg/kg)		
	Control	250	500
Males			
Hepatocellular adenoma	6/48	7/50	13/50
Hepatocellular carcinoma	14/48	13/50	22/5
Combined rates[a]	18/48 (38%)	18/50 (36%)	29/50 (58%)
Females			
Hepatocellular adenoma	2/50	4/50	6/50
Hepatocellular carcinoma	2/50	2/50	2/50
Combined rates[a]	4/50 (8%)	6/50 (12%)	8/50 (16%)

[a]Historical incidence: 335/1034 (32.4% ±9.4%).

gavage providing dose levels of 250, 500 or 1000 mg/kg/day for 13 weeks. The NOAEL was 500 mg/kg/day. At 1000 mg/kg/day, mortality and reduced body weight were reported (Bucher *et al.*, 1986). The highest dose level in the subchronic studies which produced no adverse effects was 47.62 mg/kg/day for tetramethylethylcyclohexenone and is more than 100,000 times the estimated total daily *per capita* intakes ("eaters only")* of 0.3 μg/kg for all 10 cyclopentanone and cyclohexanone derivatives used as flavour ingredients.

A chronic study on isophorone was conducted by the National Toxicology Program (NTP) during the period January 1980–January 1982. The standard NTP protocol was used with F344 rats and B6C3F$_1$ mice of both sexes. Doses were determined from the results of the 13-week subchronic toxicity study.

In the chronic study, groups of 50 male and 50 female rats and mice were each administered isophorone by corn oil gavage at doses of 0, 250 or 500 mg/kg/day for five days/week for 103 weeks (Bucher *et al.*, 1986). No clinical signs of toxicity were reported. Gavage errors accounted for a significant number of deaths (36/300) in both male and female rats.

Renal neoplasms in the male rat reported in the NTP study of isophorone. Nephropathy was noted in both test and control rats of both sexes. In test animals, increased incidence of mineral deposits in renal collecting ducts, and tubular cell hyperplasia, adenomas and adenocarcinomas were observed in male rats but not in female rats (see Table 1). Tubule mineralization was characterized by basophilic aggregates found in the medullary collecting ducts, often occurring coincidentally with lesions of chronic nephropathy.

Authors of the NTP report concluded the following: "Under conditions of these 2-year gavage studies, there is some evidence of carcinogenicity of isophorone in the male F344/N rat as shown by the occurrence of renal tubular cell adenomas and adenocarcinomas in animals given 250 or 500 mg/kg/day . . ." (Bucher *et al.*, 1986).

More recently, it has been demonstrated that renal lesions observed in the NTP study resulted from the accumulation of aggregates of $\alpha_{-2\mu}$-globulin (a low molecular weight protein synthesized in the liver) and isophorone or its metabolites in the P2 segment of the renal proximal tubule in the male F344/N rat (Borghoff *et al.*, 1990; Strasser *et al.*, 1988). The lesions do not develop in the female F344/N rat (Bucher *et al.*, 1986). Subsequent investigations have shown that the $\alpha_{-2\mu}$-globulin nephropathy found in the F344/N male rat does not develop in mammals in which the hepatic form of $\alpha_{-2\mu}$-globulin is absent (Swenberg *et al.*, 1989) such as other strains of rats (Dietrich and Swenberg, 1991), mice (Bucher *et al.*, 1986) and dogs (Webb *et al.*, 1990). There is no evidence to indicate that $\alpha_{-2\mu}$-globulin is produced in humans (Olson *et al.*, 1990). In a comprehensive review of $\alpha_{-2\mu}$-globulin nephropathy and associated renal tubule tumours produced in the male F344/N rat exposed to isophorone and other simple chemical substances (e.g. limonene, decalin and methyl isobutyl ketone), it was concluded that the F344/N rat is not an appropriate model for assessing human renal carcinogenic risk (EPA, 1991).

The Expert Panel has reviewed limonene and other substances including isophorone which produce $\alpha_{-2\mu}$-globulin nephropathy/tumours in male rats (Burdock *et al.*, 1990) and has concluded the following: "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 spontaneous initiated epithelial cells. Cell degeneration and necrosis in the P2 segment of the proximal convoluted tubule associated with accumulation of $\alpha_{-2\mu}$-globulin have been demonstrated for other compounds as well."

While humans produce low molecular weight serum proteins which are resorbed by the kidney, there is no evidence that $\alpha_{-2\mu}$-globulin is produced (Olson *et al.*, 1990). It is unknown whether any human serum proteins possess a binding site similar to that of $\alpha_{-2\mu}$-globulin. Although 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 isophorone. The accumulated evidence indicates that it is the unique anatomical, physiological and biochemical properties of the male rat kidney, especially the proximal convoluted tubule, that allows isophorone to interfere with renal processing of the strain-specific $\alpha_{-2\mu}$-globulin. Therefore, this process is not predictive of human carcinogenicity. After careful review, the FEMA Expert Panel has concluded that the mechanisms leading to the renal carcinogenic findings in the F344/N male rat are largely known and strongly indicate that the nephropathy associated with isophorone have no significance for human risk assessment.

Preputial neoplasms in the male rat reported in the NTP study. Preputial gland carcinomas were observed in 5/50 high-dose male rats and clitoral gland adenomas were reported in 2/50 low dose female rats. The preputial lesions were believed to be significant in the apparent absence of lesions in vehicle controls or low dose males, and the low historical incidence of this lesion (12/1094) in corn oil vehicle controls. However, the author emphasized that the actual incidence of lesions of the prepuce and clitoris was unknown since histological examination was performed only on animals exhibiting visible lesions (Bucher *et al.*, 1986).

The reported occurrence of macroscopic preputial tumours has been sporadic in vehicle controls in previous NTP studies (NTP, 1986). In more recent NTP studies in which histopathological examination of the prepuce was performed on all male rats, the incidence of preputial neoplasms in control rats exceeds the incidence of tumours (10%) reported in the isophorone-treated male rats. For example, incidences of preputial adenomas and carcinomas in control male rats treated only with corn oil by gavage are as high as 23% (NTP, 1994) while the incidence of preputial carcinomas has been reported to be as high as 12% (NTP, 1993a). In addition, the background incidence of clitoral gland adenomas (6 to 14%) in female rat vehicle controls in corn oil gavage studies (NTP, 1993a,b and 1994) supports the conclusion that the clitoral gland adenomas reported in two females at the low dose in the NTP study of isophorone are of no human relevance.

Hepatocellular neoplasms in male mice reported in the NTP study. Neoplastic and non-neoplastic lesions associated with administration of isophorone to male mice developed principally in the liver (see Table 2). Coagulative necrosis and hepatocytomegaly were significantly increased in dosed male mice, but decreased in female mice. There was a significant increase in the incidences of hepatocellular adenomas (13/50), carcinomas (22/50) and combined hepatocellular adenomas and carcinomas (29/50) in high-dose (500 mg/kg/day) male mice compared with the

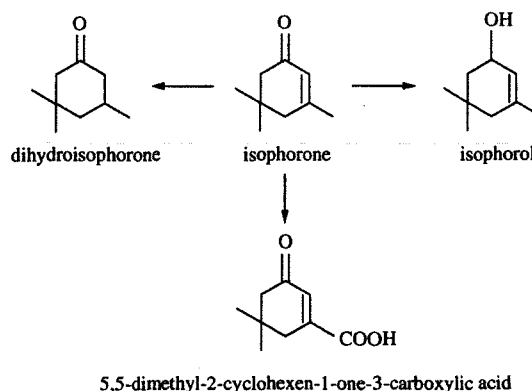5,5-dimethyl-2-cyclohexen-1-one-3-carboxylic acid

Fig. 5. Recognized metabolic fate of isophorone in rabbits. Glu = glucuronic acid.

control group (6/48, 14/48 and 18/48, respectively). There was no significant difference in the incidence of hepatocellular neoplasms between the low-dose group (250 mg/kg/day) and the control group. Female B6C3F$_1$ mice exhibited no evidence of hepatocellular neoplasms at either the 250 or 500 mg/kg/day dose level. Authors of the NTP report concluded: "For male B6C3F1 mice there was equivocal evidence of carcinogenic activity of isophorone as shown by an increased incidence of hepatocellular adenoma and carcinoma (combined) . . ." (Bucher et al., 1986).

The primary neoplastic effects observed in male mice in the NTP study were associated with the liver. The incidences of hepatocellular adenomas (6/48 and 6/50, respectively) and carcinomas (14/48 and 22/50, respectively) in the control and high-dose groups of male mice demonstrate the susceptibility and sensitivity of the B6C3F$_1$ male mouse liver to neoplastic responses. This is supported by the fact that the combined incidence of adenomas and carcinomas in control males was greater than in any group of treated females (6/50 and 8/50, respectively). These responses are consistent with the historically high levels of background hepatocellular neoplasms in B6C3F1 male mice (Maronpot and Boorman, 1982; Maronpot et al., 1987).

The Expert Panel considers that the observations of hepatic neoplasms in the NTP mouse bioassay are not relevant to the safety of isophorone in humans at low levels of intake from use as a flavour ingredient. This conclusion is based on the high incidence of spontaneous hepatocellular neoplasms (adenomas and carcinomas) in the strain of mice studied, the absence of a dose–response relationship, the lack of hepatocellular neoplastic effects in rats, and the relatively high dose levels administered, compared with intake levels from use as a flavour ingredient. The minimum dose level of 250 mg isophorone/kg in the 2-year bioassay is more than 1,000,000 times the estimated daily per capita intake ("eaters only")* of 6 ng/kg of isophorone from use as a flavour ingredient.

Neoplasms of the integumentary system in male mice reported in the NTP study. A statistically significant incidence of mesenchymal neoplasms, primarily subcutaneous fibrosarcomas, was reported in male mice at the highest dose level (500 mg/kg/day) only. The high dose group exhibited a statistically significant increase (14/50, 28%) in the incidence of mesenchymal neoplasms compared with the control group (6/48, 13%) (Bucher et al., 1986). More recent chronic bioassays in mice using corn oil as a vehicle show a high background incidence of subcutaneous fibrosarcomas. The incidence of combined subcutaneous fibrosarcomas, fibromas and fibrosarcomas in control groups of male mice have been reported to be as high as 20% (NTP, 1992b). The Expert Panel considers that the observations of mesenchymal neoplasms in the NTP mouse bioassay are not

relevant to the safety of isophorone in humans at low levels of intake. This conclusion is based on the high background incidence of spontaneous fibrosarcomas in the strain and sex of mice studied, the lack of similar neoplastic effects in rats, and the relatively high dose level (500 mg/kg/day) administered compared with intake levels from use as a flavour ingredient.

Mutagenicity and genotoxicity. Bacteria studies *in vitro* demonstrate that isophorone is not mutagenic. Isophorone at concentrations up to 10,000 mg/plate has shown no evidence of mutagenicity in *Salmonella typhimurium* strains TA98, TA102, TA104, TA1535 and TA1537 when tested with or without metabolic activation (Cheh, 1986; Mortelmans et al., 1986; Tennant et al., 1987). No increase in mutation frequency was reported when isophorone was incubated with L5178Ytk$^{+/-}$ mouse lymphoma cells with or without S-9 metabolic activation at concentrations in the range from 130 to 1300 mg/ml (McKee et al., 1987) or at cytotoxic levels (O'Donoghue et al., 1988). An increase in mutation frequency was reported in the L5178Ytk$^{+/-}$ mouse lymphoma cell assay without S-9 activation at doses up to 1600 mg/ml (McGregor et al., 1988).

There was no increase in the frequency of micronucleated polychromatic erythrocytes in the bone marrow of male or female mice administered up to 540 mg/kg isophorone by intraperitoneal injection (McKee et al., 1987).

No increase in the frequency of chromatid breaks and exchanges was reported when Chinese hamster ovary (CHO) cells were incubated with isophorone at concentrations up to 1600 mg/ml with or without metabolic activation (Tennant et al., 1987). An increase in the frequency of sister chromatid exchanges (SCE) was reported when CHO cells were incubated with isophorone at concentrations up to 1000 mg/ml without S-9 activation and only subsequent to the addition of bromodeoxyuridine (10 mM) to the incubation mixture (Gulati et al., 1989; Tennant et al., 1987).

Isophorone did not induce unscheduled DNA synthesis in rat hepatocyte cultures at concentrations up to 200 μl/ml (O'Donoghue et al., 1988; McKee et al., 1987).

The weak mutagenic response to isophorone in one of three mouse lymphoma cell forward mutation assays and the results of SCE assay in CHO cells are not compelling evidence of genotoxic potential. The inability of isophorone to induce unscheduled DNA synthesis and the lack of positive response in bacterial assays further supports the Panel's conclusion that exposure to isophorone lacks potential for interaction with DNA.

b. Carvone derivatives: chemical identity and exposure. Group III. A. 2. b. contains nine, substituted, monocyclic terpenoid ketones, secondary alcohols, and related esters that are derivatives of carvone (see Fig. A5). The 2-menthyl carbon skeletal

structure (refer to Fig. 6) is common to all carvyl derivatives. Two principal stereoisomers of carvone exist in nature, *l*-carvone and *d*-carvone. They and their corresponding secondary alcohol derivatives (e.g. *l*-carveol and *d*-carveol) are responsible for the two distinct functional uses of carvyl derivatives as flavour ingredients. The flavour and fragrance of *l*-carvone and its derivatives indicates spearmint while that of *d*-carvone and its derivatives is primarily dill or caraway (Clark, 1989).

The parent ketone carvone is commercially available as *d*-carvone, *l*-carvone and the racemic mixture (*dl*-carvone). *d*-Carvone and *l*-carvone are produced primarily by selective oxidation of *l*- and *d*-limonene, respectively. As flavouring ingredients, *l*-carvone is used mainly in spearmint flavoured products such as chewing gum, while *d*-carvone appears in dill (i.e. pickles) and caraway (i.e. alcoholic beverages) flavoured products. Significant exposure to *l*-carvone occurs from use of oral hygiene products, in spearmint flavoured toothpaste and mouthwash. Since *d*-carvone use is only 1% of *l*-carvone use, the term "carvone" is generally associated with spearmint flavour (Clark, 1989).

Carvone is used as a flavour ingredient in foods up to an average maximum level of 350 ppm in spearmint flavoured hard candy and chewing gum (see Table B9). On the basis of a reported annual volume of 52,300 kg (NAS, 1987), the estimated daily *per capita* intake ("eaters only")* of carvone is 0.17 mg/kg/day. The total *per capita* intake ("eaters only")* from use of all nine carvyl derivatives as flavour ingredients is 0.18 mg/kg/day. Carvone (i.e. mostly *l*-carvone) accounts for about 94% of the annual volume of use of all carvyl derivatives. Carveol (744 kg/year), dihydrocarveol (320 kg/year), dihydrocarvone (921 kg/year) and carvyl acetate (190 kg/year) are the only other carvyl derivatives with a significant (>100 kg/year) volume of use as flavour ingredients (NAS, 1987).

The stereoisomers of carvone (*d*, *l* and *dl*) have been reported to occur in nature in more than 100 essential oils, but usually in only trace amounts (<0.1%) (CIVO–TNO, 1989). Significant concentrations of *l*-carvone (55–75%) and *d*-carvone (20–75%) are contained in oils of *Mentha* (spearmint) and *Carum* (caraway) or *Anethum* (dill), respectively (CIVO–TNO, 1989). More than 90% of the consumption of naturally occurring carvone is from intake of *l*-carvone in oil of *Mentha* (Clark, 1989). Similarly, carveol (*d*, *l* and *dl*) and dihydrocarvone (*p*-menth-8-en-2-one) have been detected in spearmint, dill and caraway in addition to a variety of other spices, fruits and berries (CIVO–TNO, 1989). The consumption of carvone, carveol and dihydrocarvone as natural components of traditional foods has been estimated to be two to four times their respective intakes from use as flavour ingredients (Stofberg and Grundschober, 1987; Stofberg and Kirschman, 1985).

Metabolism. Carvyl derivatives metabolize like other alicyclic ketones, secondary alcohols and related esters. Ketones are reduced to the corresponding secondary alcohol and conjugated mainly with glucuronic acid. In rodents, but probably not in humans, the conjugate is excreted primarily into the bile where it may be hydrolysed to yield the free alcohol. It may then enter enterohepatic circulation and be excreted by the kidney. If a double bond is present in the molecule, the metabolite may be hydrogenated to the dihydro derivative, possibly by gut microflora.

Alternatively, alicyclic ketones containing an alkyl or alkenyl side-chain may undergo oxidation of the side-chain to form polar metabolites which are excreted as the glucuronic acid or sulfate conjugates in the urine and, to a lesser extent, in the faeces. α,β-Unsaturated ketones may be conjugated by β-addition of glutathione to the alkene.

In rabbits, carvone is reduced to yield carveol which is converted to the glucuronic acid conjugate and excreted in the urine (Fischer and Bielig, 1940). Ketone reduction occurs primarily by cytosolic carbonyl reductase, and is stereoselective to yield a mixture of diastereomeric alcohols (Leibman and Ortiz, 1973). The glucuronic acid conjugate of dihydrocarveol has been detected in the urine of rabbits (Hamalainen, 1912). Additionally, the endocyclic double bond of carvone is hydrogenated to yield 8-menthen-2-ol (dihydrocarveol), which also is excreted (Fischer and Bielig, 1940). Reduction of ketone and alkene functions has been reported for other alicyclic terpenoid ketones in rats (Madyastha and Raj, 1993) and rabbits (Dutertre-Catella *et al.*, 1978).

Also in rabbits, *dl*-carvone is metabolized by allylic oxidation of the isopropenyl side-chain to yield 9-hydroxycarvone (see Fig. 6) which is conjugated with glucuronic acid and excreted in the urine (Ishida *et al.*, 1989; Williams, 1959). As observed for other acyclic (Nishizawa *et al.*, 1987) and alicyclic ketones (Nelson *et al.*, 1992), oxidation of the side chain may be catalysed by cytochrome *P*-450 (CYP450) (Madyastha and Raj, 1990). Carvone induces cytosolic glutathione *S*-transferase activity in mouse forestomach and intestinal mucosa, and increases glutathione content in the lung, colon and intestinal mucosa (Zheng *et al.*, 1992) suggesting that the α,β-unsaturated ketone undergoes detoxication by conjugating glutathione. Carvone has been detected unchanged in the urine of humans, presumably arising from its dietary intake (Zlatkis *et al.*, 1973).

Toxicology. Carvyl derivatives generally exhibit very low oral acute toxicity. Oral LD_{50} values have been reported for seven of the nine substances and are in the range from 766 mg/kg to more than 5000 mg/kg (see Table B10).

Subchronic and chronic studies have been performed for the parent ketone carvone, which is the principal carvyl derivative used as a flavour ingredient. 10 mice per group were given *d*-carvone

Fig. 6. Recognized metabolism of carvone in rabbits.
GS = H₃N⁺CH(COO⁻)CH₂CH₂CONHCH(CH₂S)CONHCH₂COO⁻.

at doses of 0, 93, 187, 375, 750 or 1500 mg/kg in corn oil by gavage, 5 days/week for 13 weeks. Only a single mouse in each of the 750 and 1500 mg/kg dose groups survived to the end of the study. Relative liver weights were increased in male and female mice provided with 750 mg/kg/day but were not accompanied by treatment-related changes on histopathological examination (NTP, 1990).

Rats were maintained on diets containing 1000 ppm carvone (unspecified stereochemistry) for 27 to 28 weeks, 2500 ppm for 1 year or 10,000 ppm for 16 weeks (Hagan *et al.*, 1967). Dietary concentrations were calculated (FDA, 1993) to provide an average daily intake of 50 mg/kg/day for 27 to 28 weeks, 125 mg/kg/day for 1 year or 750 mg/kg/day for 16 weeks. Measurements of body weights and food intake performed weekly and haematological examination performed at 3, 6, 12 and 22 months revealed no significant difference between test and control animals. Organ weights at autopsy and histopathology showed no dose-related effects in the 27 to 28 week or 1-year studies. Depressed body weight gain and testicular atrophy were the only reported effects at the 750 mg/kg/day level in the 16-week study. However, no evidence of testicular atrophy was reported at the same dose level in the 2-year mouse bioassay (NTP, 1990).

Groups of B6C3F₁ mice were administered

d-carvone in corn oil by gavage daily at dose levels of 0, 375 or 750 mg/kg five days/week for 103 weeks. Despite the fact that exposure to carvone occurs primarily from use of *l*-carvone, the *d*-stereoisomer was chosen as the test article for the 2-year bioassay. *d*-Carvone treatment had no effect on mean body weight or survival. There was no evidence of toxicity or treatment-related non-neoplastic or neoplastic lesions in mice. The Expert Panel concurs with the following conclusion of the NTP Pathology Working Group: "Under conditions of the two-year gavage studies, there was no evidence of carcinogenic activity of *d*-carvone for male or female B6C3F1 mice administered *d*-carvone at 375 or 750 mg/kg, five days per week for two years" (NTP, 1990).

The 750 mg/kg/day dose level in the 2-year mouse gavage study that produced no carcinogenic or toxic effects is more than 1000 times the estimated total daily *per capita* intake ("eaters only")* of 0.18 mg/kg from use of all nine carvyl derivatives as flavour ingredients. The large margin of safety would accommodate any anticipated difference in toxicity between *d*-carvone used in the NTP 2-year bioassay and *l*-carvone from use as a flavour ingredient.

c. **Menthone derivatives: chemical identity and exposure.** Group III. A. 2. c. contains 10, substituted, monocyclic terpenoid ketones, secondary alcohols and related esters that are derivatives of menthone

(see Fig. A6). To varying degrees, these flavour ingredients exhibit a physiological cooling effect and minty character. The 3-menthyl carbon skeletal structure (see menthol, Fig. 7) is common to these alicyclic substances which include menthol and its stereoisomer *neo*-menthol, menthone and its stereoisomer *iso*-menthone, 3 menthyl esters, a glyceryl menthyl ether and two dehydromenthyl derivatives.

Although the parent alcohol menthol has eight possible stereoisomers, the most abundant stereoisomer in nature is *l*-menthol. Only the *l* and *dl* forms are important commercially. Menthone with two stereocenters has four possible stereoisomers. Two stereoisomers, *l*-menthone and *d-iso*-menthone, predominate in nature.

The menthyl derivatives are used at highest concentration in peppermint flavoured chewing gum and hard candy. Menthol is used as a flavour ingredient in these foods up to average maximum levels of 2300 and 2100 ppm, respectively, and in other foods up to 185 ppm. *d,l-iso*-Menthone is used in confectionery frosting up to 60 ppm and in chewing gum up to 600 ppm (see Table B11).

The annual volumes of menthol (54,400 kg/year, 76.8%), menthone (13,300 kg/year, 18.8%) and menthyl acetate (2940 kg/year, 4.1%) account for more than 99% of the total annual volume (approx. 70,800 kg/year) of all menthone derivatives used as flavour ingredients (NAS, 1987). The estimated total daily *per capita* intake ("eaters only")* of all 10 menthone derivatives from use as flavour ingredients is 0.22 mg/kg. The *per capita* intake of menthol by "cool mint" eaters who consume 125 g (approx. $\frac{1}{4}$ lb) of candy containing 2000 ppm menthol is about 4 mg/kg.

Menthyl derivatives occur naturally in a wide variety of foods. *l*-Menthol (50–60%) and menthone (15–30%) are primary constituents of peppermint oil. They also occur in spearmint oil, raspberries, and rum, nutmeg and cocoa (CIVO–TNO, 1989). The consumption of menthol (principally the *l*-isomer), menthone and menthyl acetate as natural components of peppermint oil and spearmint oil have been estimated to be approximately four to 10 times their intake from use as flavour ingredients (Stofberg and Grundschober, 1987; Stofberg and Kirschman, 1985). All but two of the menthone derivatives have been reported to occur naturally in foods (CIVO–TNO, 1989).

Metabolism. In general, menthol is metabolized by conjugation with glucuronic acid or oxidation to various polyols and hydroxyacids. The metabolites of menthol are eliminated in the urine and faeces either unchanged or conjugated with glucuronic acid. The parent ketone menthone is metabolized like other alicyclic ketones and is reduced to the corresponding secondary alcohol, *neo*-menthol, which is metabolized and eliminated by pathways similar to the ones utilized by its stereoisomer menthol.

The metabolic fate of menthone derivatives has been studied in humans and other animals. The vast majority of either a 1000 mg (Quick, 1928), 750 mg (Eisenberg *et al.*, 1955) or 10–20 mg (Atzl *et al.*, 1972) oral dose of menthol administered to volunteers was eliminated as the glucuronide. In two separate studies involving a total of 19 male and female volunteers, the glucuronic acid conjugate of menthol was detected in the urine following oral administration of a 180 mg dose of peppermint oil (Kaffenberger and Doyle, 1990). In rabbits, orally administered menthol is conjugated with glucuronic acid and eliminated in the urine (Deichmann and Thomas, 1943; Quick, 1924; Williams, 1938 and 1939). The glucuronide is a minor urinary excretion product in dogs, suggesting that oxidation of menthol is more important in this species (Williams, 1938). In rats, the vast majority of orally administered menthol is eliminated in either the urine or faeces as the glucuronic acid conjugate or various oxidation products (Madyastha and Srivatsan, 1988a; Yamaguchi *et al.*, 1994). Menthol glucuronide formed in the liver passes into bile with subsequent elimination or entry into enterohepatic circulation. The biliary route of metabolism of menthol is more important in rodents and dogs compared with humans and rabbits. Oxidation products of menthol include *p*-menthane-3,8-diol primarily, *p*-menthane-3,9-diol and 3,8-dihydroxy-*p*-menthane-7-carboxylic acid (Madyastha and Srivatsan, 1988a; Yamaguchi *et al.*, 1994) (see Fig. 7). Additional oxidation metabolites have been identified including a primary alcohol, a triol and hydroxyacids (Yamaguchi *et al.*, 1994). Results of an *in vitro* study using rat liver microsomes suggest that oxidation of menthol is mediated by CYP450. Rats receiving repeated oral doses of menthol for 3 days exhibited increased activity of hepatic microsomal CYP450 and NADPH-cytochrome *P*-450 reductase (Madyastha and Srivatsan, 1988a).

l-Menthone given to rabbits (Neubauer, 1901; Williams, 1940) was stereoselectively reduced to *d-neo*-menthol (see Fig. 8); the stereoisomer *d-iso*-menthone was reduced to *d-iso*-menthol (Williams, 1940). The vast majority of a 1000 mg/kg dose of *d-neo*-menthol given to rabbits by stomach tube was eliminated in the urine as the glucuronic acid conjugate (Williams, 1940). As with other alicyclic terpenoids such as menthol, *dl-iso*-menthone may also undergo oxidation of the alkyl ring substituents. Data on structurally related alicyclic terpenoids suggests that oxidation occurs preferentially on the isopropyl or methyl substituent and not on the cyclohexane ring (Yamaguchi *et al.*, 1994).

Toxicology. Menthone derivatives exhibit very low acute oral toxicity. Oral LD_{50} values have been reported for seven of the 10 substances and are in the range from 940 to 7870 mg/kg (see Table B12).

The toxicology of the menthone derivatives is supported by subchronic and chronic oral studies that have been performed with menthol in mice and

Fig. 7. Recognized metabolism of menthol in animals.

rats. No adverse effects were reported when rats were fed menthol in the diet for 5.5 weeks providing doses of 100 or 200 mg/kg (FAO, 1967). In a 28-day gavage study, rats were provided with 0, 200, 400 or 800 mg menthol/kg/day. A significant increase in absolute and relative liver weights and vacuolation of hepatocytes were reported at all dose levels (Thorup et al., 1983a). These results were not confirmed by long-term studies in which menthol was administered in the diet.

Fig. 8. Recognized metabolism of l-menthone in rabbits.

In a subchronic study of dl-menthol, 10 F344 rats and 10 B6C3F₁ mice of each sex were maintained on diets containing 930, 1870, 3750, 7500 or 15,000 ppm for 13 weeks (NCI, 1978). A dose-related increase in mortality was reported in female mice only. All animals were autopsied, and histological examination was conducted for controls, highest-dose groups, and some animals in the second highest-dose groups only. A slight increase in the incidence of interstitial nephritis was observed in male rats in the highest-dose group. The incidence of perivascular lymphoid hyperplasia and interstitial nephritis was slightly increased in female mice in the two highest-dose groups.

In a carcinogenicity study for 103 weeks, groups of 50 male and 50 female F344 rats or B6C3F₁ mice were maintained on diets containing dl-menthol at levels of either 3750 or 7500 ppm for rats and 2000 or 4000 ppm for mice (NCI, 1978). Dietary concentrations were calculated (FDA, 1993) to provide corresponding average daily intake levels of 187 mg/kg or 375 mg/kg for rats and 300 mg/kg or 600 mg/kg for mice. No neoplastic lesions related to administration of menthol were observed in any group compared with controls.

The highest dose levels of 600 and 375 mg/kg/day menthol that produced no adverse effects in mice and rats, respectively, are more than 10,000 times the total daily per capita intake ("eaters only")* of 0.22 mg/kg

9-hydroxyisopulegol   **Isopulegol**   glucuronic acid conjugate
                                                     of isopulegol

Fig. 9. Proposed metabolism of *iso*-pulegol in humans.

from the use of all menthone derivatives as flavour ingredients. These dose levels are about 100 times the *per capita* intake of about 4 mg/kg menthol by "cool mint" eaters.

d. *iso*-Pulegone derivatives: chemical identity and exposure. Group III. A. 2. d. contains five substituted monocyclic terpenoids, including three ketones, a secondary alcohol and a related ester (see Fig. A7). The 3-menthyl carbon skeleton is common to all five *iso*-pulegone derivatives. Pulegone and *p*-mentha-1,4(8)-dien-3-one have an isopropylidene side-chain $((CH_3)_2C=$; i.e. a 4(8)-alkene) while *iso*-pulegone, *iso*-pulegol and *iso*-pulegol acetate have a 2-isopropenyl side-chain $((CH_2=C-CH_3$; i.e. an 8(9)-alkene). Pulegone has two possible stereoisomers; $(R)-(+)$-pulegone is the predominant form in nature. Both the (R)- form and racemic mixture are sold commercially.

The *iso*-pulegone derivatives exhibit a minty, herbaceous odour. They are used as flavour ingredients in foods up to average maximum levels of 35 ppm (see Table B13). The annual volume of isopulegol is 1370 kg/year and accounts for more than 97% of the total annual volume of use (approx. 1406 kg/year) of all *iso*-pulegone derivatives as flavour ingredients (NAS, 1987). The estimated total daily *per capita* intake ("eaters only")* of all five *iso*-pulegone derivatives is about 4.5 μg/kg from use as flavour ingredients.

Four of the five *iso*-pulegone derivatives have been reported to occur naturally in foods such as the essential oils of peppermint, pennyroyal, spearmint and citrus peel (CIVO–TNO, 1989). Significant concentrations of *iso*-pulegol have been reported in orange peel oil (4000 ppm) and mandarin peel oil (80 ppm). The (R) isomer of pulegone is a major constituent (35–52%) of pennyroyal oil (mentha pulegium). The intake of $(R)-(+)$-pulegone and *p*-mentha-1,4(8)-dien-3-one from intake of food is more than100 and more than10 times, respectively, their intake from use as flavour ingredients (Stofberg and Grundschober, 1987; Stofberg and Kirschman, 1985).

Metabolism. Alicyclic secondary alcohols are conjugated with glucuronic acid and excreted primarily in the urine. Ketones may be reduced to the corresponding secondary alcohols. Additionally,

activated alkyl substituents and activated ring positions (e.g. allylic position) may be oxidized to form polar metabolites which are readily conjugated and excreted. Side-chain oxidation of pulegone and *p*-mentha-1,4(8)-dien-3-one may lead to products (e.g. 9-hydroxypulegone) which have unique structural features that promote formation of reactive metabolites which may be cytotoxic at relatively high cellular concentration. α,β-Unsaturated ketones and reactive metabolites may also be conjugated with glutathione.

The glucuronic acid conjugate of *iso*-pulegol is excreted in the urine of rats administered the corresponding ketone pulegone (Madyastha and Raj, 1993; Thomassen *et al.*, 1991). Glucuronide conjugation of *iso*-pulegol, catalysed by uridine diphosphate-glucuronosyltransferase, has been demonstrated in rat and guinea pig liver microsomes *in vitro* (Boutin *et al.*, 1985). In addition to glucuronide formation, monocyclic terpenoid alcohols and ketones (e.g. α-terpineol, sobrerol and carvone) undergo allylic oxidation of isopropenyl substituents to yield diols, hydroxyketones and hydroxyacids, which are readily excreted in the urine and faeces (Ishida *et al.*, 1989; Madyastha and Srivatsan, 1988b; Ventura *et al.*, 1985; Yamaguchi *et al.*, 1994). It is expected that *iso*-pulegol will be detoxified in humans by conjugation of the alcohol with glucuronic acid and allylic oxidation of the isopropenyl substituent to yield 9-hydroxy-isopulegol (see Fig. 9).

Pulegone has extremely low use (15 kg/year) as a flavour ingredient (NAS, 1987), but is a major constituent of pennyroyal oil (CIVO–TNO, 1989) which, at relatively high dose levels (> 100 mg/kg), has been associated with liver and lung toxicity in animals (see Toxicology section). The metabolic fate of pulegone has been extensively studied in the rat. Pulegone is metabolized through four primary pathways: the ketone function is reduced to yield pulegol (I); the exocyclic alkene is oxidized to yield 2,8-dihydroxymenthone (II); the tertiary ring carbon (C_5) is hydroxylated to yield 5-hydroxypulegone (III); and, in the predominant pathway, the isopropylidene substituent undergoes allylic oxidation to yield 9-hydroxypulegone (IV) (Madyastha and Raj, 1993) (see Fig. 10). Metabolites of pulegone are rapidly conjugated and excreted into the bile of

rats. Pulegone and its metabolites form glucuronic acid and glutathione conjugates as well as mixed glutathionyl–glucuronide conjugates which are excreted in the urine and faeces (Thomassen *et al.*, 1991).

In the major pathway, pulegone undergoes regiospecific allylic hydroxylation (Nelson *et al.*, 1992) to form 9-hydroxypulegone which is the common intermediate for formation of several metabolites. In a secondary detoxication pathway, 9-hydroxypulegone is oxidized to 9-carboxypulegone (i.e. 5-methyl-2-(1-methyl-1-carboxyethylidene) cyclo-

hexanone) (Moorthy *et al.*, 1989) which, in part, cyclizes to a hydroxylactone (see Fig. 10) or undergoes oxidation and hydration to yield polar hydroxyacids (Madyastha and Raj, 1993). In a secondary pathway, 9-hydroxypulegone cyclizes through formation of an intramolecular hemiketal which then undergoes dehydration to yield mainly menthofuran (Gordon *et al.*, 1987; Madyastha and Raj, 1993).

Menthofuran is then converted to a variety of unreactive and reactive metabolites. Stable excretable metabolites include those derived from ring cleavage

Fig. 10. Recognized metabolism of pulegone in rats.

(geranic acid and neronic acid), fragmentation and aromatization (*p*-cresol) (Madyastha and Raj, 1993) and hydration and cyclization (mintlactone and *iso*-mintlactone) (Nelson *et al.*, 1992; Thomassen *et al.*, 1992) (see Fig. 10). In an important intoxication pathway, menthofuran is the proximate toxic metabolite which is further oxidized by hepatic CYP450 to yield a γ-ketoenal, an ultimate toxic metabolite (McClanahan *et al.*, 1989; Madyastha and Raj, 1990 and 1993; Nelson *et al.*, 1992; Thomassen *et al.*, 1991). It has been proposed that the γ-ketoenal forms by a 2,3-epoxyfuran intermediate (Nelson *et al.*, 1992; Thomassen *et al.*, 1992). The rate of formation of the γ-ketoenal in mouse, rat and human hepatic microsomes with menthofuran as the substrate (Thomassen *et al.*, 1992) is about five to 10 times faster than the rate with pulegone as the substrate (McClanahan *et al.*, 1989). These data suggest that the γ-ketoenal is formed from pulegone through menthofuran.

Menthofuran-derived metabolites account for 70–80% of the hepatic microsomal metabolism of pulegone *in vitro*. Menthofuran and other minor metabolites of pulegone, to some extent, conjugate with glutathione (Thomassen *et al.*, 1990).

Toxicology. The very low oral acute toxicity of *iso*-pulegol and its acetate ester is demonstrated by rat oral LD_{50} values of 1200 mg/kg and more than 5000 mg/kg, respectively. The oral acute toxicity of pulegone is demonstrated by a rat oral LD_{50} of 470 mg/kg (see Table B14).

The acute toxicity of pulegone in humans has been associated with the ingestion of pennyroyal oil which contains pulegone as a major constituent. The clinical pathology of ingestion of large doses of pennyroyal oil by humans is characterized by massive centrilobular necrosis, pulmonary oedema, internal bleeding and weight loss (Sullivan *et al.*, 1979). At one time pennyroyal oil was used as an ineffective abortifacient. Ingestion of approximately 24 g (Sullivan *et al.*, 1979) or 500 mg/kg (Gordon *et al.*, 1982) of pennyroyal oil in attempted abortions caused death while women ingesting approximately 7.5 ml of the oil have recovered, apparently without liver damage (Gunby, 1979). The pathogenesis of hepatotoxicity in mice, rats, and humans associated with ingestion of pennyroyal oil is reported to be initiated by CYP450 oxidation of pulegone (Nelson *et al.*, 1992).

Animal studies have mostly shown hepatotoxicity, although pulegone is also reported to produce necrosis of pulmonary bronchiolar epithelial cells following intraperitoneal and oral administration to mice at dose levels greater than 100 mg/kg (Gordon *et al.*, 1982). Pulegone induces hepatic injury in Swiss–Webster, Balb/c (Gordon *et al.*, 1982) and ddY mice (Mizutani *et al.*, 1987) and in rats (Moorthy *et al.*, 1989) at dose levels above 100 mg/kg. The resulting lesions in these studies were characterized by centrilobular necrosis of hepatocytes, similar to that observed in humans. Significant (1.4–20 times control values) dose-related increases in serum hepatic alanine aminotransferase (ALT) activity were reported with oral doses of 100 to 400 mg/kg in rats (Moorthy *et al.*, 1989) and intraperitoneal doses 300 mg/kg or more in mice (Gordon *et al.*, 1982; Mizutani *et al.*, 1987). The severity of hepatic necrosis was associated in a dose-related fashion with an increase in serum ALT activity in mice (Gordon *et al.*, 1982). Glucose-6-phosphatase activity was significantly decreased, suggesting damage to the hepatic endoplasmic reticulum (Moorthy *et al.*, 1989).

In a single-dose acute toxicity study, mice were administered isopulegone, piperitenone, isopulegol or pulegone in corn oil by intraperitoneal injection (Gordon *et al.*, 1982). At a dose level of 400 mg/kg nine of 16 pulegone treated animals died within 24 hours and five of six survivors exhibited extensive (>6 to >50% of hepatocytes) necrosis of liver tissue. At the same dose level, no mortalities were observed after treatment with isopulegol, isopulegone or piperitenone. Less severe liver necrosis was observed in five of 20 isopulegone-treated mice and three of 10 piperitenone-treated mice. Isopulegol treatment was not associated with any hepatotoxic effects. These results indicate that pulegone is significantly more hepatotoxic than other pulegone derivatives (see Mechanism of Hepatotoxicity).

Pulegone was administered to rats by gavage for 28 days providing dose levels of 0, 20, 80 and 160 mg/kg/day (Thorup *et al.*, 1983a). At dose levels of 80 and 160 mg/kg/day, pulegone was reported to cause histopathological changes in the liver and dose-related alterations in the brain which appeared as cyst-like spaces in the white matter. Two additional short-term studies originating from the same laboratory on peppermint oil (Thorup *et al.*, 1983b) and menthone (Madsen *et al.*, 1986) also included reports of "cyst-like spaces" in the white matter of the cerebellum. These effects were not observed in rats treated with 0, 200, 400 and 800 mg/kg/day menthol (Thorup *et al.*, 1983a).

The two pathologists on the FEMA Expert Panel reviewed the original rat brain histology slides from the three studies (Madsen *et al.*, 1986; Thorup *et al.*, 1983a,b). As a result of this review and taking into account additional data, the following observations were made:

(1) In each of the three studies the report of "cyst-like spaces" in the white matter of the cerebellum were not accompanied by any evidence of cellular reaction in tissue adjacent to the spaces. Moreover, there was no indication of neurotoxicity with pulegone administration.

(2) Attempts to reproduce the "cyst-like spaces" in rat white cerebellar tissue, even when higher dose levels of peppermint oil were administered to the same strain of rat (Mengs and Stotzem, 1989), have

failed to confirm results of the three original studies (Madsen *et al.*, 1986; Thorup *et al.*, 1983a,b).

(3) A study with peppermint oil completed in 1992 using a brain perfusion method did not reveal any evidence of "cyst-like spaces" in the cerebellum of any of the rats (Olsen, 1994). Following re-examination of the slides and consideration of all available data, the Expert Panel concluded that the weight of evidence indicates that the rat cerebellar "cyst-like spaces" found with peppermint oil, pulegone and menthone were artefacts arising from inadequate tissue fixation procedures.

The NOAEL of pulegone in rats is reported to be 20 mg/kg/day and is more than 100,000 times the daily *per capita* intake ("eaters only")* of 50 ng/kg of pulegone from use as a flavour ingredient. The 20 mg/kg/day dose level is more than 1000 times the daily *per capita* intake ("eaters only")* of 4.5 μg/kg from use of all five *iso*-pulegone derivatives as flavour ingredients. This NOAEL is likely to be a significant underestimate for *iso*-pulegol and its derivatives due to the markedly increased toxicity of pulegone relative to *iso*-pulegol.

Mechanism of hepatotoxicity. According to Gordon *et al.* (1982), the genesis of pulegone-induced liver toxicity apparently requires the presence of the α-isopropylidene ketone unit: "Reduction of either the ketone group, as in pulegol, or the isopropylidene double bond, as in menthone completely eliminates the hepatotoxic and lung toxic response. Isomerization of the double bond to the alicyclic position, as in *iso*-pulegone, significantly decreases the toxic potential; isomerization to an endocyclic position, as in piperitone, eliminates the organ toxic response. A combination of exocyclic and endocyclic double bonds, as in piperitenone, also decreases the toxic potential. Removal of the isopropylidene unit, as in R-(+)-3-methylcyclohexanone, eliminates hepatotoxicity and lung toxicity, whereas removal of the methyl group, as in cyclohexylidenylcyclohexanone, only decreases the toxic response."

Stereochemistry also plays a part; (S)-(−)-pulegone exhibited similar hepatotoxic effects in mice, but at doses two to three times that of (R)-(+)-pulegone (Nelson *et al.*, 1992). The (α-isopropylidene substituent present in pulegone is necessary for the *in vitro* destruction of CYP450 (Moorthy *et al.*, 1991).

The mechanism of hepatotoxicity has been related to CYP450-induced metabolism of pulegone. The extent of pulegone-induced hepatic necrosis increased with phenobarbital pretreatment but not with β-naphthoflavone pretreatment, indicating that the formation of a toxic metabolite is apparently mediated by CYP450 of the phenobarbital class (Gordon *et al.*, 1987). Hepatotoxicity was significantly mitigated by simultaneous treatment of animals with inhibitors of CYP450 monooxygenases (e.g. SKF-525A, metyrapone, piperonyl butoxide,

cobaltous chloride and CS_2) (Gordon *et al.*, 1987; Mizutani *et al.*, 1987; Moorthy *et al.*, 1989) and potentiated by pretreatment with activators of CYP450 (Moorthy *et al.*, 1989). The effect of CYP450 inducers and inhibitors on hepatotoxicity suggests that pulegone itself is not a hepatotoxin, but is oxidized by CYP450 to a toxic metabolite (Nelson *et al.*, 1992). On the basis of a comparison of the plasma ALT levels in pulegone- and menthofuran-treated animals, it has been suggested that the menthofuran pathway accounts for a majority of the hepatotoxicity related to exposure to pulegone (Thomassen *et al.*, 1988). In this pathway menthofuran is converted to a γ-ketoenal, the principal toxic metabolite formed by the 2,3-epoxyfuran intermediate. Other reactive metabolites (i.e. *p*-cresol, piperitenone) may also play a minor role in the toxicity of (R)-(+)-pulegone. It has been proposed that the binding of the reactive γ-ketoenal or epoxyfuran intermediate to cellular proteins, specifically including CYP450, is directly related to the observed hepatotoxicity (Gordon *et al.*, 1982; Moorthy *et al.*, 1989; Thomassen *et al.*, 1991) and pulmonary toxicity (Gordon *et al.*, 1982) of R-(+)-pulegone in animals. Pulegone treatment results in a dose-dependent decrease in rat hepatic CYP450 and haem content both *in vivo* and *in vitro* (Madyastha *et al.*, 1985; Moorthy *et al.*, 1989 and 1991). Repeated administration of pulegone to rats results in a marked decrease in hepatic CYP450 and haem content, but no significant changes are observed in the activities of either cytochrome B_5 or NADPH-cytochrome c reductases. It has been suggested that pulegone metabolites specifically deactivate CYP450 by modifying the prosthetic haem or apoprotein of the enzyme (Madyastha *et al.*, 1985; Moorthy *et al.*, 1991).

No changes in CYP450 or haem content of rats were noted at the dose of 100 mg/kg but effects were observed at dose levels of 200 and 400 mg/kg (Moorthy *et al.*, 1989). This is consistent with the very slight increase in plasma GPT (1.4 times control value at 100 mg/kg *v.* about 20 times control values at 400 mg/kg) activity at the 100 mg/kg dose level. At the same dose levels pulegone treatment resulted in no significant change in the levels of CYP450 and in the activities of cytochrome B_5 or NADPH-cytochrome c reductase in kidney microsomes, suggesting that a sufficient concentration of the reactive metabolite responsible for the effects in the liver may not be present in the kidney to elicit effects on CYP450 (Moorthy *et al.*, 1989). If changes in plasma ALT activity, CYP450 content, and haem content are used as markers for the hepatotoxicity of pulegone, dose levels of pulegone less than 100 mg/kg/day would not be considered hepatotoxic. It appears that at dose levels less than 100 mg/kg, cellular concentrations of pulegone and its metabolites are sufficiently detoxicated by conjugation with glutathione and glucuronic acid.

Pulegone and its primary and secondary metabolites form glucuronic acid and glutathione conjugates as well as mixed glutathionyl–glucuronide conjugates which protect against menthofuran-induced toxicity. When mice were given hepatotoxic doses of pennyroyal oil or (R)-(+)-pulegone, hepatic glutathione levels decreased significantly (about 75%) within 3 hours followed by a rapid increase in plasma hepatic aminotransferase activity (i.e. ALT). When mice were pretreated with diethylmaleate to decrease hepatic glutathione levels, the hepatotoxic response to (R)-(+)-pulegone markedly increased (Gordon *et al.*, 1982). Glutathione forms conjugates with pulegone, reduced pulegone and oxidized pulegone which has been tentatively identified as a glutathione conjugate of menthofuran. The other two glutathione conjugates apparently undergo subsequent glucuronidation to yield novel mixed glutathionyl glucuronide conjugates (Thomassen *et al.*, 1991). On the basis of these observations it has been proposed that glutathione conjugation plays a major part in the detoxication of the CYP450 bioactivated-pulegone metabolite (i.e. menthofuran or the (γ-ketoenal) (Thomassen *et al.*, 1991).

It is anticipated that the metabolic intoxication pathway of pulegone demonstrated in animals may also exist in humans resulting in formation of menthofuran and other reactive metabolites. At high dose levels, menthofuran is a proximate hepatotoxic substance. However, if the concentration of toxic metabolites of pulegone is not sufficient to deplete hepatocellular concentrations of glutathione (5–10 mM) (Armstrong, 1987; Sies *et al.*, 1983), hepatotoxicity would not be observed. Therefore, the levels of pulegone and its reactive metabolites from use as a flavour ingredient (50 ng/kg/day) and as a natural component of food (1–2 mg/kg/day) (Higley, 1994; Stofberg and Kirschman, 1985) are expected to be insufficient for depleting the hepatocellular concentration of glutathione and therefore would not be hepatotoxic.

3. Bicyclic and macrocyclic

Chemical identity and exposure. Group III. A. 3. comprises 21 bicyclic and macrocyclic terpenes including seven ketones, five secondary alcohols and nine related esters (see Fig. A8). 16 of the substances are monoterpenes with a bridged bicyclic structure resembling the parent substance, *d*-camphor. With the exception of verbenol and 2(10)-pinen-3-ol (i.e. pinocarveol), the common carbon skeleton is two cyclopentane rings joined at the 1- and 4-positions (i.e. bicyclo(2.2.1)heptane system) with an oxygen function on the ring. Verbenol and 2(10)-pinen-3-ol have a 4- and 6-membered ring. The chemical structure of these bicyclic terpenes is associated with the distinctive burning, bitter, fresh taste of *d*-camphor that is reminiscent of mint. The odour is described as "camphoraceous". Several of the substances may have pine or citrus notes.

The other five substances are ketones: nootkatone, dihydronootkatone and 7-methyl-4,4a,5,6-tetrahydro-2(3*H*)naphthalenone have a common carbon skeleton of two fused cyclohexane rings and exhibit the flavour of grapefruit; muscone (C_{15}) and civettone (C_{17}) are macrocyclic ketones which exhibit a powerful musky odour.

The bridged bicyclic terpenes (16) are used as flavour ingredients up to average maximum use levels of 30 ppm (see Table B15). Only four of these bicyclic terpenes have annual volumes greater than 50 kg from use as flavour ingredients, as reported in the most recent survey (NAS, 1987). The remaining 12 have annual volumes less than 5 kg. *d*-Camphor and *iso*-bornyl acetate have the highest volumes in this group, 907 and 210 kg/year, respectively, followed by *l*-borneol and fenchyl alcohol which each have volumes of 90 kg/year. On the basis of the most recent reported annual volumes (NAS, 1987) for all 16 bridged bicyclic terpenes used as flavour ingredients, the total daily *per capita* intake ("eaters only")* is estimated to be 5 µg/kg. *d*-Camphor accounts for approximately 60% of the total *per capita* intake ("eaters only") of the 16 camphor derivatives in group III. A. 3.

Nootkatone and its two fused ring derivatives are used as flavour ingredients up to an average maximum level of 20 ppm. The *total* annual volume of their use is less than 500 kg, as reported in the most recent survey (NAS, 1987) (see Table B15). The annual volumes of nootkatone and 7-methyl-4,4a,5,6-tetrahydro-2(3*H*)-naphthalenone are 50 and 350 kg, respectively. The estimated total daily *per capita* intake ("eaters only")* is 1.5 mg/kg of the three nootkatone derivatives from use as flavour ingredients. The powerful odour of musk exhibited by the two macrocyclic ketones limits their average maximum use to levels below 0.05 ppm. The combined reported annual volume of their use as flavour ingredients is less than 1 kg (NAS, 1987), which corresponds to a total daily *per capita* intake ("eaters only")* of 2 ng/kg.

12 of the 16 bicyclic terpenes have been reported to occur in nature (CIVO–TNO, 1989). Most occur naturally in various essential oils and are commonly found in spices. *l*-Borneol and its esters have been identified in more than 250 plants, herbs, leaves or bark. Consumption ratios have been estimated for *d*-camphor, *l*-borneol, fenchyl alcohol and bornyl acetate and are 4, 10, 10 and more than 100, respectively (Stofberg and Grundschober, 1987; Stofberg and Kirschman, 1985).

Nootkatone and dihydronootkatone occur naturally in a variety of citrus fruits, mainly in grapefruit. Nootkatone has been reported at highest concentration in grapefruit peel oil (2000 ppm) and grapefruit juice (6 ppm) (CIVO–TNO, 1989). Its consumption as a natural component of grapefruit is estimated to be more than 10 times its intake from use as a flavour ingredient (Stofberg and Grundschober,

Fig. 11. Recognized metabolism of camphor in humans.

1987; Stofberg and Kirschman, 1985). Both macrocyclic ketones muscone and civettone occur naturally as secretions from the glands of animals. The *cis* form of civettone, a component of the secretion of the special glands of Ethiopian civet cats, and muscone are used mainly in fragrances (Burdock, 1994).

Camphor is readily used in over-the-counter (OTC) medications as a rubefacient in products such as Vicks Vaporub at concentrations of 0.3–20%. It is also used as an analgesic in infants to treat teething pain and in dentistry as an antiseptic. The levels of camphor used in OTC medications are significantly higher than the levels of *d*-camphor used as a flavour ingredient. The plasma concentration of camphor in a human who fully recovered following ingestion of 300 mg/kg camphor was 1.7 mg/litre (Koppelman *et al.*, 1979) which is approximately 100,000 times the estimated plasma concentration of 17 ng/litre expected to result from the daily *per capita* intake ("eaters only")* of 3 μg/kg *d*-camphor from use as a flavour ingredient.

Metabolism. The structurally related bridged bicyclic terpenes fall into one of three classes of compounds which governs their mode of metabolism; ketones, secondary alcohols, or esters. Compared with short-chain aliphatic (Bosron and Ting-Kai, 1980) or monocyclic (Dutertre-Catella *et al.*, 1978; Fischer and Bielig, 1940; Madyastha and Raj, 1993) ketones which are reduced to the corresponding secondary alcohol, the bicyclic ketones exhibit greater lipophilicity and steric hindrance of the carbonyl function and therefore are expected to be poor substrates for cytosolic reducing enzymes. The predominant mode of metabolic detoxication for

bridged monoterpenoid ketones is CYP50-mediated ring hydroxylation to yield polar, excretable metabolites.

In humans, ingested camphor undergoes ring hydroxylation to yield 3-, 5-, 8- and 9-hydroxycamphor, 5-ketocamphor, and the carboxylic acid of either 8- or 9-hydroxycamphor which are excreted in the urine unchanged or conjugated with glucuronic acid. A small amount is exhaled in expired air (Koppel *et al.*, 1982) (see Fig. 11). Hydroxylation products of *d*-camphor have been reported in liver fractions of rats and rabbits (Leibman and Ortiz, 1973), and in the urine of dogs and rabbits after oral administration (Leibman and Ortiz, 1973; Robertson and Hussain, 1969). Similarly, the urinary metabolites of dogs fed *d*-fenchone were 4- and 5-hydroxyfenchone and *p*-apofenchone-3-carboxylic acid (Reinartz and Zanke, 1936). Camphor induced members of the cytochrome P450IIB sub-family in rat liver, probably *P*-450b and/or *P*-450e (Austin *et al.*, 1988). NADPH-dependent reduction of *d*-camphor which occurs stereoselectively to yield borneol in the rabbit (Robertson and Hussain, 1969; Leibman and Ortiz, 1973) may occur in humans to a small extent.

Bicyclic secondary alcohols such as borneol are rapidly detoxified in humans (Wagreich *et al.*, 1941) and dogs (Miller *et al.*, 1933; Pryde and Williams, 1934; Quick, 1927) by conjugation with glucuronic acid and excretion in the urine (see Fig. 12). Fenchyl alcohol in olive oil given to rabbits by stomach tube was excreted in the urine as the glucuronic acid conjugate (Hamalainen, 1912). The glucuronic acid conjugates of verbenol and 2(10)-pinen-3-ol were

Glu = glucuronic acid

Fig. 12. Recognized metabolism of borneol in humans.

detected in the urine of rabbits orally administered α-pinene (Ishida *et al.*, 1981). Increased levels of β-glucuronidase were reported in several tissues of dogs orally administered borneol (Fishman, 1940).

The induction of cytochrome *P*4501A1 in rats injected with borneol was observed in rat liver microsomes (Hiroi *et al.*, 1995) suggesting that oxidation may occur to a small extent. Verbenol contains an alkene function and may undergo oxidation of the allylic methyl group in a secondary metabolic pathway similar to that observed for the structurally related terpenoid *trans*-sobrerol (Ventura *et al.*, 1985). In addition, the highly-strained four-membered ring present in verbenol and 2(10)-pinen-3-ol may undergo cleavage to monocyclic secondary alcohols similar to that reported for structurally related ring-strained bicyclic aldehydes (Ishida *et al.*, 1989).

The nine esters of bicyclic alcohols and linear or branched-chain aliphatic carboxylic acids are expected to be hydrolysed in humans to the component alcohol and carboxylic acid. Metabolism of the bicyclic secondary alcohol components is described above. The carboxylic acid components would undergo complete metabolism in recognized biochemical pathways (Voet and Voet, 1990). Data on structurally related esters and the metabolism of bornyl acetate support the hydrolysis of bicyclic terpenes.

Ester hydrolysis is catalysed by classes of enzymes recognized as carboxylesterases or esterases (Heymann, 1980), the most important of which are the B-esterases. In mammals, these enzymes occur in most tissues throughout the body (Anders, 1989; Heymann, 1980) but predominate in the hepatocytes (Heymann, 1980). Rabbits orally administered bornyl acetate excreted borneol as the glucuronic acid conjugate (Williams, 1959).

Fused ring and macrocyclic ketones are detoxified by pathways similar to the bridged bicyclic substances. Activated (e.g. tertiary and allylic positions) ring positions and ring substituents are oxidized primarily by non-specific CYP450s to introduce additional polar functionalities into the molecule. The resulting metabolites are then excreted mainly in the urine.

Nootkatone-13,14-diol was isolated as a neutral metabolite from the urine of rabbits given large (6 g) oral doses of nootkatone, and was probably formed by epoxidation of the exocyclic isopropenyl substituent (Asakawa *et al.*, 1986) (see Fig. 13).

Structurally related fused ring ketones also undergo oxidation of ring positions which are remote from the ketone function (Asakawa *et al.*, 1986; Devon and Scott, 1972). For instance, the tricyclic α,β-unsaturated ketone cyclocolorenone is metabolized to yield two hydroxyketone metabolites in which C_9 (methylene) and C_{10} (methine) ring positions are hydroxylated (Asakawa *et al.*, 1986) (see Fig. 14). Similar pathways of oxidation of the double bond, ring positions and ring alkyl substituents are expected in humans.

Toxicology. The very low oral acute toxicity of bicyclic terpenes has been demonstrated in animals. Oral LD_{50} values were reported for 11 of the 16 substances in this group and range from more than 5000 mg/kg to more than 10,000 mg/kg for *iso*-bornyl acetate (see Table B16). Data from several clinical reports of camphor poisoning in humans from OTC medications indicate that camphor is toxic when ingested at dose levels greater than 50 mg/kg (Geller *et al.*, 1984) producing vomiting, cardiac stimulation, hallucinations and convulsions. A dose of 50 mg/kg ingested by a pregnant woman caused foetal death (Riggs *et al.*, 1965) and 700 mg produced death in children (Baselt and Cravey, 1989). Adults have tolerated doses up to 18 g with treatment (Koppelman *et al.*, 1979).

The oral acute toxicity of the three fused ring ketones and two macrocyclic ketones has been studied and is very low. Oral LD_{50} values range from 1220 mg/kg to more than 5000 mg/kg (see Table B16).

The subchronic toxicity of the bicyclic terpenes is supported by subchronic studies conducted for the acetate ester of *iso*-borneol. In addition, three limited studies have been reported for borneol, *d*-fenchone, and an essential oil which contains *d*-camphor. *iso*-Bornyl acetate was administered to rats in corn oil by gavage for 13 weeks at doses of 0, 15, 90 or 270 mg/kg/day (Gaunt *et al.*, 1971). At the highest dose level, nephrotoxicity was observed in males including increased kidney weight, vacuolation and exfoliation of renal tubular cells, decreased renal concentrating ability, and increased water intake.

Fig. 13. Recognized metabolism of nootkatone in rabbits.

Fig. 14. Recognized metabolism of cyclocolorenone in rabbits.

Increased relative liver and caecum weights were also observed in males and females at the highest dose. Some nephrotoxicity was reported at the 90 mg/kg/day dose. The NOAEL was 15 mg/kg/day and is more than 1000 times the total daily *per capita* intake ("eaters only")* of 5 µg/kg from use of the 16 camphor derivatives as flavour ingredients.

In a limited study designed to study glucuronic acid detoxication, three dogs given 526 mg borneol/kg/day by stomach tube for 31 days showed no ill-effects (Miller *et al.*, 1933). Toxic effects were observed in a second group administered borneol for approximately 3 months at dose levels that were gradually increased to 1300 mg/kg/day and a third group of animals that were fasted and fed borneol. *d*-Fenchone was orally administered to dogs for 16 days (Rimini, 1901). A dose level of 750 mg/kg produced temporary tremors and convulsions, and 1400 mg/kg produced convulsions and eventual death. Doses of 210–420 mg/kg did not result in toxic effects.

In a limited 8-week toxicity study using controls (Skramlik, 1959), 250 mg salvia oil/kg/day (i.e. clary sage oil) which is estimated to contain approximately 30% camphor (Millet *et al.*, 1981) was well tolerated when given to white rats by oral administration. When the dose was increased to 500 mg/kg/day, some convulsing was observed. When increased to 1000 mg/kg/day most animals died, and all animals died when the level was increased to 1250 mg/kg/day. The NOAEL of salvia oil of 250 mg/kg/day corresponds to approximately 75 mg camphor/kg/day and is more than 10,000 times the total daily *per capita* intake ("eaters only")* of 5 µg/kg from use of all bicyclic terpenes as flavour ingredients.

Subchronic and chronic feeding studies have been performed for alicyclic α,β-unsaturated ketones structurally related to nootkatone derivatives, such as carvone (FEMA No. 2249; see Fig. A5). No effects were reported when rats were maintained on diets containing 1000 ppm carvone (unspecified stereochemistry) for 27–28 weeks or 2500 ppm for 1 year (Hagan *et al.*, 1967). Dietary concentrations were calculated (FDA, 1993) to provide an average daily intake of 50 mg/kg/day for 27–28 weeks or 125 mg/kg/day for 1 year.

Groups of B6C3F₁ mice were administered *d*-carvone in corn oil by gavage daily at dose levels of 0, 375 or 750 mg/kg five days/week for 103 weeks.

Carvone treatment had no effect on mean body weight or survival. There was no evidence of toxicity or treatment-related non-neoplastic or neoplastic lesions in mice (NTP, 1990). The 125 mg/kg/day dose level in the rat dietary study and the 750 mg/kg/day dose level in the mouse gavage study that produced no adverse effects are more than 10,000 times the daily *per capita* intake ("eaters only")* of 1.5 µg/kg from use of the three nootkatone derivatives as flavour ingredients. The large margin of safety would accommodate any anticipated difference in toxicity between these alicyclic α,β-unsaturated ketones.

B. Functional group on the side-chain

Chemical identity and exposure. Group III. B. contains 21 ketones and secondary alcohols with the functional group on the side-chain (see Fig. A9). This group includes 16 ionone derivatives, four damascone derivatives, and one substance that is structurally related to damascone. The substances in this group contain a 2,6,6-trimethylcyclohexyl carbon skeleton. An alkyl ring substituent 4–7 carbon atoms in length is located at the C_1 position; these acyclic longer-chain alkyl groups have an oxygenated functional group and varying degrees of unsaturation. With the exception of γ-ionone, each of these substances has at least one endocyclic double bond. The ketone function of the damascones is positioned a to the ring, while the hydroxyl or carbonyl group of the ionones is γ to the ring. The skeletal structure of ionones and damascones is present in β-carotene and vitamin A. Both groups of flavours are known for their floral, fruity odours. Ionones have varying strengths and characters of violet fragrances, while the damascones, commonly known as rose ketones, have the odour of roses.

α-Ionone and β-ionone also have the highest average maximum levels of use which are 60 and 275 ppm, respectively, in chewing gum only (see Table B17). On the basis of the most recent reported annual volumes (NAS, 1987) of the ionones and damascones, the estimated total daily *per capita* intake levels ("eaters only")* are 5 and 0.25 µg/kg , respectively, from use as flavour ingredients. The estimated combined daily *per capita* intake ("eaters only")* of α- and β-ionone is 4.1 µg/kg (Table B17). The use of these two ionones accounts for approximately 83% of the total annual volume of all ionone derivatives. The only members of this group with significant (>100 kg/year) volume of usage as

flavour ingredients are α-ionone (768 kg/year), β-ionone (549 kg/year) and allyl α-ionone (130 kg/year) (NAS, 1987).

More than half (13/21) of the group III. B. substances have been reported to occur as natural components of food (CIVO–TNO, 1989). α-Ionone and β-ionone have been detected in raspberries, carrots, roasted almonds, fruits and herbs (CIVO–TNO, 1989). Highest intake of α- and β-ionone occurs from the consumption of carrots (approx. 70 and 90%, respectively). Consumption ratios for substances in this group are reported in Table B17.

Metabolism. In animals, ketones with the functional group on the side-chain are reduced to the corresponding secondary alcohol followed by glucuronide conjugation. In addition, allylic hydroxylation of the ring or methyl substituents may occur. The resulting hydroxy metabolites may conjugate with glucuronic acid or undergo further oxidation to the corresponding ketone (Williams, 1959). Combinations of these detoxication reactions result in the formation of multiple metabolites which are subsequently excreted in the urine and faeces. Michael addition of reduced glutathione at the β-position of α,β-unsaturated ketones may also occur in a minor pathway (Armstrong, 1991; Portoghese *et al.*, 1989).

In rabbits, orally-administered α-ionone is primarily metabolized to 5-hydroxy derivatives by ring oxidation (Prelog and Wursch, 1951). Orally-administered β-ionone is primarily metabolized to 4-oxo (i.e. 4-keto) and 4-hydroxy derivatives by ring oxidation of β-ionone and β-ionol. The latter forms through reduction of the ketone function in rabbits

(see Fig. 15) (Ide and Toki, 1970; Prelog and Meier, 1950). Unchanged β-ionone and glucuronide conjugates of 4-oxo-β-ionol and dihydro-4-oxo-β-ionol were also detected (Ide and Toki, 1970). The allylic oxidation products of β-ionone, β-ionol and their exocyclic dihydro metabolites also were detected in rabbits (Bielig and Hayasida, 1940). β-Ionone has been found to induce biphenyl 4-hydroxylase, glucuronyl transferase, 4-nitrobenzoate reductase and CYP450 in rats following 3-day administration by either intraperitoneal injection or food (Parke and Rahman, 1969).

The metabolism of ionones is expected to be similar in humans. This is supported by human metabolism studies of retinoids and carotenoids such as *cis*-13-retinoic acid (i.e. isotretinoin) and β-carotene, respectively, which possess ionone fragments. The primary blood and biliary metabolites of isotretinoin following oral administration to humans include the glucuronide conjugates of isotretinoin (Kraft *et al.*, 1991) and the allylic oxidation product 4-oxo-isotretinoin (Kraft *et al.*, 1991; Vane *et al.*, 1990). Both metabolites were also observed in the blood and bile of cynomolgus monkeys provided with isotretinoin by the oral route (Kraft *et al.*, 1991). Allylic hydroxylation and glucuronic acid conjugation of the methyl ring substituent also occurs in humans (Vane *et al.*, 1990) (see Fig. 16).

The occurrence of β-ionone in carrots arises from oxidation and cleavage of the 9′,10′-double bond of β-carotene. β-Ionone may be present endogenously as a minor metabolite in the animal metabolism of β-carotene. The latter is oxidized by carotenoid

Fig. 15. Recognized metabolism of β-ionone in rabbits.

β -ionone fragment

13-*cis*-retinoic acid
(isotretinoin)

4-hydroxy-13-*cis*-retinoic acid
(4-hydroxy-isotretinoin)

4-oxo-13-*cis*-retinoic acid
(4-oxo-isotretinoin)

16-hydroxy-13-*cis*-retinoic acid
(16-hydroxy-isotretinoin)

18-hydroxy-13-*cis*-retinoic acid
(18-hydroxy-isotretinoin)

Fig. 16. Recognized metabolism of 13-*cis*-retinoic acid in humans and monkeys.

dioxygenase(s) and cleaved at the 15-15' (central) double bond to yield two molecules of vitamin A (retinal) (Simpson and Chichester, 1981). Cleavage of the 9'-10' double bond may also occur to yield β-ionone and 10-apo-β-carotenals, and is suggested by the presence of 10'-apo-β-carotenal in rat liver following oral administration of β-carotene (Sharma et al., 1977).

Toxicology. The acute oral toxicity of ionone and damascone derivatives is very low as demonstrated by oral LD₅₀ values reported for 16 of the 21 substances in the group. Rodent oral LD₅₀ values range from more than 1220 to 8800 mg/kg (see Table B18).

A mixture of 60% α-ionone and 40% β-ionone was administered to rats in the diet at concentrations of 0, 1000, 2500 or 10,000 ppm for 17 weeks (Hagan et al., 1967). These dietary concentrations are calculated (FDA, 1993) to provide daily dose levels of 0, 50, 125 or 500 mg/kg, respectively. Dose-related slight to moderate swelling of the parenchymal cells of the liver was reported. Fatty infiltration of liver parenchymal cells was also noted in rats fed 13-115 mg α- and β-ionone/day for 5-9 days (Shillinger, 1950) which corresponds to a calculated (FDA, 1993) dose level of approximately 32-288 mg/kg.

β-Ionone was administered to rats in the diet in cottonseed oil providing an average daily intake of 11.6 mg/kg to males and 13.1 mg/kg to females (Oser et al., 1965) for 12 weeks. Haematological examinations conducted at weeks 6 and 12 revealed normal values. Liver and kidney weights at autopsy were normal, and histopathology revealed no dose-related effects. A similar 12-week study in which rats were orally provided 11.4 mg β-ionone/kg/day resulted in no adverse effects (Bar and Griepentrog, 1967).

Sprague-Dawley rats were fed a mixture of α- and β-ionone in the diet providing an average daily intake of 10 or 100 mg/kg for a minimum of 90 days

(BIBRA, 1983). At the 100 mg/kg/day dose level, increased liver weight, reduced weight gain and food consumption, decreased serum glucose concentrations, increased water intake, and mild renal function changes were reported. A slightly increased liver weight was reported at the 10 mg/kg/day dose level, but was not accompanied by any evidence of histopathology. The change in liver weight was judged (BIBRA, 1983) to be of no toxicological significance. The 10 mg/kg/day NOAEL is more than 1000 times the estimated total daily per capita intake ("eaters only")* of 5.0 μg/kg from use of all 16 ionone derivatives as flavour ingredients.

1-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-2-buten-1-one, commonly referred to as β-damascone, was administered to rats in the diet in microcrystalline cellulose providing an average daily intake of 2.38 mg/kg to males and 2.35 mg/kg to females for 13 weeks (Posternak et al., 1975). An increase in food intake, liver weights and kidney weights of test females was reported but was not accompanied by evidence of histopathology. No toxicologically significant haematological variations were noted. The dose level of 2.3 mg/kg for β-damascone that produced no adverse effects is about 1000 times the estimated total daily per capita intake ("eaters only")* of 0.25 μg/kg from use of all five damascone derivatives as flavour ingredients.

IV. Tertiary alcohols and related esters

Chemical identity and exposure. Group IV. comprises 13 tertiary alcohols and related esters (see Fig. A10). These terpene substances are used as flavour ingredients up to an average maximum level of 85 ppm (see Table B19). More than 95% of the total intake of this group of substances is associated with consumption of α-terpineol (70%) and terpinyl acetate (25%). On the basis of the annual volumes reported for all 13 tertiary alcohols and related esters

Fig. 17. Recognized metabolism of α-terpineol in rats.

used as flavour ingredients (NAS, 1987), the estimated total daily *per capita* intake ("eaters only")* is 25 μg/kg.

Nine of the 13 substances have been reported to occur naturally in foods, including a wide variety of fruits and fruit juices, vegetables and spices. 4-Carvomenthenol occurs in nutmeg oil at 250 ppt and α-terpineol occurs in lime peel oil at 91 ppt (CIVO–TNO, 1989).

Metabolism. In humans and animals, tertiary alcohols primarily conjugate with glucuronic acid and are excreted in the urine and faeces (Horning *et al.*, 1976; Ventura *et al.*, 1985; Williams, 1959). If the alicyclic tertiary alcohol contains an alkyl or alkenyl substituent, it may undergo oxidation of the side-chain to form polar metabolites which may also be excreted free or in the conjugated form. In rats, α-terpineol undergoes oxidation of the allylic methyl group to yield the corresponding carboxylic acid which, to a small extent, is hydrogenated to yield the corresponding saturated carboxylic acid (see Fig. 17). α-Terpineol orally administered to rats increased the liver microsomal *P*-450 content and activity of NADPH-cytochrome c reductase (Madyastha and Srivatsan, 1988b) suggesting that its oxidation is mediated by CYP450.

In a minor pathway, the endocyclic alkene of α-terpineol is oxidized to yield a triol metabolite, which has been reported in humans following inadvertent oral ingestion of a pine oil disinfectant containing α-terpineol (Horning *et al.*, 1976).

In a metabolism study using the structurally related *trans*-sobrerol in humans, dogs and rats, 10 metabolites were isolated in urine, eight of which were isolated in human urine. Two principal modes of metabolism were observed, including allylic oxidation of the ring positions and alkyl substituents and conjugation of the alcohol functions with glucuronic acid. These metabolic patterns are common modes of converting tertiary (Ventura *et al.*, 1985) and secondary (Yamaguchi *et al.*, 1994) terpenoid alcohols to polar metabolites which are easily excreted in the urine and faeces (see Fig. 18). Menthol forms similar oxidation and conjugation products in rats (Yamaguchi *et al.*, 1994).

Bicyclic tertiary alcohols are relatively stable *in vivo*, eventually leading to conjugation and excretion. In addition, cases of conversion to a monocyclic structure have been documented (Williams, 1959). In rabbits, the bicyclic tertiary alcohol β-santenol

Fig. 18. Observed metabolism of *trans*-sobrerol in rats, dogs and humans. **Metabolite was isolated in human urine. Glu = glucuronic acid.

Fig. 19. Proposed metabolism of 2-ethyl-1,3,3-trimethyl-2-norbornanol in humans. Glu = glucuronic acid.

(1,7-dimethyl[2.2.1]-2-exo-bicycloheptanol; see Fig. A10) is conjugated with glucuronic acid (Williams, 1959). The bicyclic alcohol thujyl alcohol (see Fig. A10) was fed to rabbits and excreted as the glucuronic acid conjugate. In addition, the mono-cyclic diol p-menthane-2,4-diol was reported as a product of ring cleavage (Williams, 1959). The metabolism of 2-ethyl-1,3,3-trimethyl-2-norbornanol, based on information from structurally related bicyclic alcohols, is presented in Fig. 19.

Esters undergo hydrolysis to yield the corresponding alcohol and acid. Esters of α-terpineol would yield α-terpineol and a simple acyclic aliphatic acid. The acid component would undergo complete metabolism to carbon dioxide and water (Voet and Voet, 1990). One ester, methyl 1-acetoxycyclohexyl ketone, is expected to hydrolyse to acetic acid and methyl 1-hydroxycyclohexyl ketone, which would be excreted as the glucuronic acid conjugate.

Toxicology. Tertiary alcohols and related esters exhibit very low acute toxicity as demonstrated by LD_{50} values in the range from 1300 to 5075 mg/kg which have been reported for eight of the 13 substances in this group (see Table B20).

No adverse effects were reported when rats were fed terpinyl acetate providing dietary levels up to 10,000 ppm for 20 weeks (Hagan et al., 1967). This dietary level is calculated (FDA, 1993) to result in an average daily intake of 500 mg/kg and is more than 10,000 times the estimated total daily per capita intake ("eaters only")* of 25 μg/kg from use of all 13 tertiary alcohols and related esters as flavour ingredients.

Since terpenoid alcohols such as α-terpineol, trans-sobrerol and menthol are metabolized to glucuronic acid conjugates, polyols and hydroxy acids through similar pathways, the chronic toxicity of menthol (FEMA No. 2665; see Fig. A6) provides a basis for the estimation of the toxic potential of α-terpineol, terpinyl acetate and other related substances. In a subchronic feeding study of dl-menthol, 10 F344 rats and 10 B6C3F₁ mice of each sex were provided with dose levels of 930, 1870, 3750,

7500 and 15,000 ppm for 13 weeks (NCI, 1978). A dose-related increase in mortality was reported in female mice only. All animals were autopsied, and histological examination was conducted for controls, highest-dose groups, and some animals in the second highest-dose groups only. A slight increase in the incidence of interstitial nephritis was observed in male rats in the highest dose group. The incidence of perivascular lymphoid hyperplasia and interstitial nephritis was slightly increased in mice in the highest dose groups.

In a carcinogenicity study, groups of F344 rats and B6C3F₁ mice (50 animals of each sex and species per group) were administered dl-menthol in feed for 103 weeks providing dose levels of 3750 or 7500 ppm for rats, and 2000 or 4000 ppm for mice (NCI, 1978). Dietary concentrations were calculated (FDA, 1993) to provide corresponding average daily intake levels of 187 mg/kg or 375 mg/kg for rats and 300 mg/kg or 600 mg/kg for mice. No neoplastic lesions related to administration of menthol were observed in any group compared with controls (NCI, 1978). The dose levels of terpinyl acetate and menthol that produced no adverse effects are more than 10,000 times the estimated total daily per capita intake ("eaters only")* of 25 μg/kg from the intake of all 13 tertiary alcohols and related esters from use as flavour ingredients.

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VI. Appendix A

Primary alcohols, aldehydes, carboxylic acids and related esters	Figure
Monocyclic, unsubstituted	A1
Monocyclic and polycyclic, substituted	A2

Ketones, secondary alcohols and related esters functional group on the ring

Monocyclic, unsubstituted	A3
Monocyclic, substituted	

Cyclopentanone and cyclohexanone derivatives	A4
Carvone derivatives	A5
Menthone derivatives	A6
iso-pulegone derivatives	A7
Bicyclic and macrocyclic	A8

Ketones, secondary alcohols and related esters functional group on the side-chain	A9
Tertiary alcohols and related esters	A10

1.   Cyclohexanecarboxylic acid
      FEMA No. 3531
      CAS No. 98-89-5

5.   Cyclohexaneacetic acid
      FEMA No. 2347
      CAS No. 5292-21-7

2.   Methyl cyclohexanecarboxylate
      FEMA No. 3568
      CAS No. 4630-82-4

6.   Ethyl cyclohexanepropionate
      FEMA No. 2431
      CAS No. 10094-36-7

3.   Ethyl cyclohexanecarboxylate
      FEMA No. 3544
      CAS No. 3289-28-9

*Allyl cyclohexanepropionate
FEMA No. 2026
CAS No. 2705-87-5

4.   Cyclohexaneethyl acetate
      FEMA No. 2348
      CAS No. 5452-75-5

*Structurally related substance.

Fig. A1. Primary alcohols, aldehydes, carboxylic acids and related esters: unsubstituted monocyclics.
*Structurally related substance.

1. (2,2,3-Trimethylcyclopent-3-en-
1-yl)acetaldehyde
FEMA No. 3592
CAS No. 4501-58-0

2. *cis*-5-Isopropenyl-*cis*-2-methylcyclo-
pentan-1-carboxaldehyde
FEMA No. 3645
CAS No. 55253-28-6

3. Campholene acetate
FEMA No. 3657
CAS No. 36789-59-0

4. α-Campholenic alcohol
FEMA No. 3741
CAS No. 1901-38-8

5. *p*-Menth-1-en-9-al
FEMA No. 3178
CAS No. 29548-14-9

6. 1-*p*-Menthen-9-yl acetate
FEMA No. 3566
CAS No. 17916-91-5

7. *p*-Menth-1,8-dien-7-al
FEMA No. 3557
CAS No. 2111-75-3

8. *p*-Mentha-1,8-dien-7-ol
FEMA No. 2664
CAS No. 536-59-4

9. *p*-Mentha-1,8-dien-7-yl acetate
FEMA No. 3561
CAS No. 15111-96-3

Fig. A2 (*Continued overleaf*).

10.   1,2,5,6-Tetrahydrocuminic acid
      FEMA No. 3731
      CAS No. 71298-42-5

14.   2-Formyl-6,6-dimethylbicyclo
      (3.1.1.) hept-2-ene
      FEMA No. 3395
      CAS No. 564-94-3

11.   2,6,6-Trimethylcyclohexa-1,3-
      dienyl methanal
      FEMA No. 3389
      CAS No. 116-26-7

15.   Myrtenol
      FEMA No. 3439
      CAS No.   515-00-4

12.   2,6,6-Trimethyl-1-cyclohexen-
      1-acetaldehyde
      FEMA No. 3474
      CAS No. 472-66-2

16.   Myrtenyl acetate
      FEMA No. 3765
      CAS No. 1079-01-2

13.   2,6,6-Trimethyl-1&2-cyclohexen-
      1-carboxaldehyde
      FEMA No. 3639
      CAS No. 432-25-7

(1)   (2)

17.   2-Hydroxymethyl-6,6-dimethyl-
      bicyclo [3.1.1]hept-2-enyl formate
      FEMA No. 3405
      CAS No. 72928-52-0

Fig. A2 (*Continued opposite*).

18.   Santalol (α & β)
      FEMA No. 3006
      CAS No. 77-42-9

α   β

19.   Santalyl acetate
      FEMA No. 3007
      CAS No. 1323-00-8

Fig. A2. Primary alcohols, aldehydes, carboxylic acids and related esters: substituted monocyclics and polycyclics.

1.   Cyclohexyl formate
     FEMA No. 2353
     CAS No. 4351-54-6

2.   Cyclohexyl acetate
     FEMA No. 2349
     CAS No. 622-45-7

3.   Cyclohexyl propionate
     FEMA No. 2354
     CAS No. 6222-35-1

4.   Cyclohexyl butyrate
     FEMA No. 2351
     CAS No. 1551-44-6

5.   Cyclohexyl isovalerate
     FEMA No. 2355
     CAS No. 7774-44-9
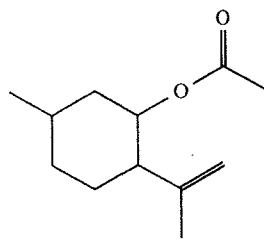
Cyclohexanol[*]

OH

Methylcyclohexanol[†]

H₃C   OH
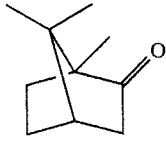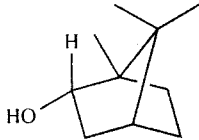
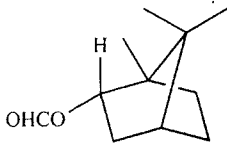Fig. A3. Ketones, secondary alcohols and related esters with a functional group on the ring: unsubstituted monocyclics. *Component alcohol of cyclohexyl esters. †Structurally related substance.

1.   1-Methyl-1-cyclopenten-3-one
      FEMA No. 3435
      CAS No. 2758-18-1

6.   3-Methyl-5-propyl-2-cyclo-
      hexen-1-one
      FEMA No. 3577
      CAS No. 3720-16-9

2.   2-Hexylidenecyclopentanone
      FEMA No. 2573
      CAS No. 17373-89-6

7.   2,2,6-Trimethylcyclohexanone
      FEMA No. 3473
      CAS No. 2408-37-9

3.   *iso*-Jasmone
      FEMA No. 3552
      CAS No. 11050-62-7

8.   Isophorone
      FEMA No. 3553
      CAS No. 78-59-1

9.   2-*sec*-Butylcyclohexanone
      FEMA No. 3261
      CAS No. 14765-30-1

4.   3-Methyl-2-(2-pentenyl)-2-
      cyclopenten-1-one
      FEMA No. 3196
      CAS No. 488-10-8

10.   Tetramethylethylcyclohexenone
      FEMA No. 3061
      CAS No. 999999-25-9

5.   3-Methyl-2-cyclohexen-1-one
      FEMA No. 3360
      CAS No. 1193-18-6

Fig. A4. Ketones, secondary alcohols and related esters with a functional group on the ring: substituted monocyclics cyclopentanone and cyclohexanone derivatives.

1. *p*-Menthan-2-one
   FEMA No. 3176
   CAS No. 499-70-7

2. *p*-Menthan-2-ol
   FEMA No. 3562
   CAS No. 499-69-4

3. *p*-Menth-8-en-2-one
   FEMA No. 3565
   CAS No. 7764-50-3

4. Dihydrocarveol
   FEMA No. 2379
   CAS No. 619-01-2

5. Dihydrocarvyl acetate
   FEMA No. 2380
   CAS No. 20777-49-5

6. Carvone
   FEMA No. 2249
   CAS No. 99-49-0

7. Carveol
   FEMA No. 2247
   CAS No. 99-48-9

8. Carvyl acetate
   FEMA No. 2250
   CAS No. 97-42-8

9. Carvyl propionate
   FEMA No. 2251
   CAS No. 97-45-0

Fig. A5. Ketones, secondary alcohols and related esters with a functional group on the ring: substituted monocyclics carvone derivatives.

1. Menthol
    FEMA No. 2665
    CAS No. 89-78-1

6. Menthyl *iso*-valerate
    FEMA No. 2669
    CAS No. 16409-46-4

2. *d-neo*-Menthol
    FEMA No. 2666
    CAS No. 20752-34-5

7. *l*-Menthyl lactate
    FEMA No. 3748
    CAS No. 59259-38-0

3. Menthone
    FEMA No. 2667
    CAS No. 89-80-5

8. *p*-Menth-1-en-3-ol
    FEMA No. 3179
    CAS No. 491-04-3

4. *dl-iso*-Menthone
    FEMA No. 3460
    CAS No. 491-07-6

9. *d*-Piperitone
    FEMA No. 2910
    CAS No. 6091-50-5

5. Menthyl acetate
    FEMA No. 2668
    CAS No. 16409-45-3

10. 3-*l*-Menthoxypropane-1,2-diol
    FEMA No. 3784
    CAS No. 87061-04-9

Fig. A6. Ketones, secondary alcohols and related esters with a functional group on the ring: substituted monocyclics methone derivatives.

1. Isopulegone
    FEMA No. 2964
    CAS No. 29606-79-9

4. Pulegone .
    FEMA No. 2963
    CAS No. 89-82-7

2. Isopulegol
    FEMA No. 2962
    CAS No. 89-79-2

5. *p*-Mentha-1,4(8)-dien-3-one
    FEMA No. 3560
    CAS No. 491-09-8

3. Isopulegyl acetate
    FEMA No. 2965
    CAS No. 57576-09-7

Fig. A7. Ketones, secondary alcohols and related esters with a functional group on the ring: substituted monocyclics iso-pulegone derivatives.

1.   *d*-Camphor
     FEMA No. 2230
     CAS No. 464-49-3

7.   *Iso*-Borneol
     FEMA No. 2158
     CAS No. 124-76-5

2.   *l*-Borneol
     FEMA No. 2157
     CAS No. 507-70-0

8.   *iso*-Bornyl formate
     FEMA No. 2162
     CAS No. 1200-67-5

3.   Bornyl formate
     FEMA No. 2161
     CAS No. 7492-41-3

9.   *iso*-Bornyl acetate
     FEMA No. 2160
     CAS No. 125-12-2

4.   Bornyl acetate
     FEMA No. 2159
     CAS No. 76-49-3

10. *iso*-Bornyl propionate
     FEMA No. 2163
     CAS No. 2756-56-1

5.   Bornyl valerate
     FEMA No. 2164
     CAS No. 7549-41-9

11. *iso*-Bornyl *iso*-valerate
     FEMA No. 2166
     CAS No. 7779-73-9

6.   Bornyl *iso*-valerate
     FEMA No. 2165
     CAS No. 76-50-6

12. *d*-Fenchone
     FEMA No. 2479
     CAS No. 4695-62-9

Fig. A8 (*Continued opposite*)

13. Fenchyl alcohol
FEMA No. 2480
CAS No. 1632-73-1

18. Dihydronootkatone
FEMA No. 3776
CAS No. 20489-53-6

14. 1,3,3-Trimethyl-2-norbornanyl
acetate
FEMA No. 3390
CAS No. 13851-11-1

19. 7-Methyl-4,4a,5,6-tetrahydro-
2(3H)naphthalenone
FEMA No. 3715
CAS No. 34545-88-5

15. Verbenol
FEMA No. 3594
CAS No. 473-67-6

20. 3-Methyl-1 cyclopentadecanone
FEMA No. 3434
CAS No. 541-91-3

16. 2(10)-Pinen-3-ol
FEMA No. 3587
CAS No. 5947-36-4

17. 4,4a,5,6,7,8-Hexahydro-4,4a-
dimethyl-3(2H)naphthalenone
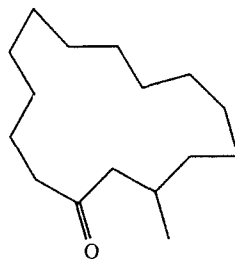nootkatone
FEMA No. 3166
CAS No. 4674-50-4

21. Cycloheptadeca-9-en-1-one
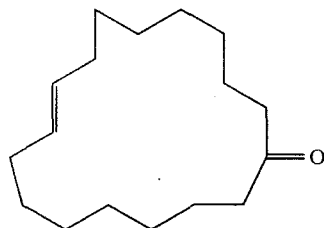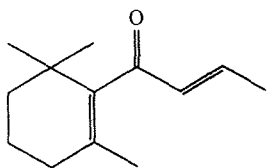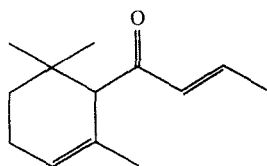FEMA No. 3425
CAS No. 542-46-1

Fig. A8. Ketones, secondary alcohols and related esters with a functional group on the ring: bicyclic and macrocyclic substances.

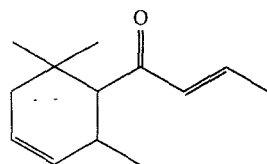1. 4-[(2,6,6)-Trimethylcyclohex-1-
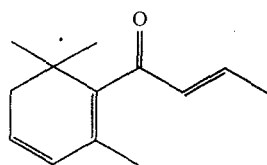enyl]but-2-en-4-one
β-Damascone
FEMA No. 3243
CAS No. 23726-92-3

6. α-Ionone
FEMA No. 2594
CAS No. 127-41-3

2. α-Damascone
FEMA No. 3659
CAS No. 43052-87-5

7. β-Ionone
FEMA No. 2595
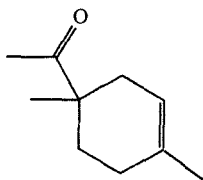CAS No. 14901-07-6

3. δ-Damascone
FEMA No. 3622
CAS No. 57378-68-4

8. γ-Ionone
FEMA No. 3175
CAS No. 79-76-5

4. 4-(2,6,6-Trimethyl-2-cyclohexa-
1,3-dienyl)but-2-en-4-one
FEMA No. 3420
CAS No. 23696-85-7

9. α-Ionol
FEMA No. 3624
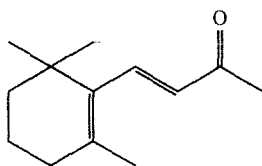CAS No. 25312-34-9

5. 1,4-Dimethyl-4-acetyl-1
cyclohexene
FEMA No. 3449
CAS No. 43219-68-7

10. β-Ionol
FEMA No. 3625
CAS No. 22029-76-1

Fig. A9 (*Continued opposite*)

11. Dihydro-α-ionone
FEMA No. 3628
CAS No. 31499-72-6

12. Dihydro-β-Ionone
FEMA No. 3626
CAS No. 17283-81-7

13. Dihydro-β-ionol
FEMA No. 3627
CAS No. 3293-47-8

14. Dehydrodihydroionone
FEMA No. 3447
CAS No. 20483-36-7

15. Dehydrodihydroionol
FEMA No. 3446
CAS No. 57069-86-0

16. Methyl-α-ionone
FEMA No. 2711
CAS No. 127-42-4

17. Methyl-β-ionone
FEMA No. 2712
CAS No. 127-43-5

18. Methyl-δ-ionone
FEMA No. 2713
CAS No. 7784-98-7

19. Allyl-α-ionone
FEMA No. 2033
CAS No. 79-78-7

20. α-Irone
FEMA No. 2597
CAS No. 79-69-6

21. α-iso-Methylionone
FEMA No. 2714
CAS No. 127-51-5

Fig. A9. Ketones, secondary alcohols and related esters with a functional group on the side-chain.

1.   α-Terpineol
      FEMA No. 3045
      CAS No. 98-55-5

5.   Terpinyl butyrate
      FEMA No. 3049
      CAS No. 2153-28-8

2.   Terpinyl *formate*
      FEMA No. 3052
      CAS No. 2153-26-6

6.   Terpinyl *iso*-butyrate
      FEMA No. 3050
      CAS No. 7774-65-4

3.   Terpinyl acetate
      FEMA No. 3047
      CAS No. 80-26-2

7.   Terpinyl *iso*-valerate
      FEMA No. 3054
      CAS No. 1142-85-4

4.   Terpinyl propionate
      FEMA No. 3053
      CAS No. 80-27-3

8.   *p*-Menth-3-en-1-ol
      FEMA No. 3563
      CAS No. 586-82-3

Fig. A10 (*Continued opposite*).

9. *p*-Menth-8-en-1-ol
    (β-Terpineol)
    FEMA No. 3564
    CAS No. 138-87-4

12. 2-Ethyl-1,3,3-trimethyl-2-
    norbornol
    FEMA No. 3491
    CAS No. 18368-91-7

10. 4-Carvomenthenol
    FEMA No. 2248
    CAS No. 562-74-3

13. Methyl 1-acetoxycyclohexyl ketone
    FEMA No. 3701
    CAS No. 52789-73-8

11. 4-Thujanol
    FEMA No. 3239
    CAS No. 546-79-2

Thujyl alcohol*   β-Santenol*

Fig. A10. Tertiary alcohols and related esters. *Structurally related substance.

VII. Appendix B

Primary alcohols, aldehydes, carboxylic Table
acids and related esters

Monocyclic, unsubstituted
Summary of usage data   B1
Summary of metabolism and toxicity data   B2

Monocyclic and polycyclic, substituted
Summary of usage data   B3
Summary of metabolism and toxicity data   B4

Ketones, secondary alcohols and related
esters functional group on the ring
Monocyclic, unsubstituted
Summary of usage data   B5
Summary of metabolism and toxicity data   B6

Monocyclic, substituted
Cyclopentanone and cyclohexanone
derivatives
Summary of usage data   B7
Summary of metabolism and toxicity data   B8

Carvone derivatives
Summary of usage data   B9
Summary of metabolism and toxicity data   B10

Menthone derivatives
Summary of usage data   B11
Summary of metabolism and toxicity data   B12

***Iso*-Pulegone derivatives**
Summary of usage data   B13
Summary of metabolism and toxicity data   B14

Bicyclic and macrocyclic
Summary of usage data   B15
Summary of metabolism and toxicity data   B16

Ketones, secondary alcohols and related esters
functional on the side-chain
Summary of usage data   B17
Summary of metabolism and toxicity data   B18

Tertiary alcohols and related esters
Summary of usage data   B17
Summary of metabolism and toxicity data   B18

Table B1. Summary of usage data for primary alcohols, aldehydes, carboxylic acids and related esters: unsubstituted monocyclics

Substance	FEMA No.	CAS No.	Annual volume 1982 (kg)	Annual volume 1987 (kg)	Most recent per capita intake (μg/kg/day)[1]	Approx. max use level (ppm)	Natural occurrence in food[2]	Consumption ratio[3]
1. Cyclohexanecarboxylic acid	3531	98-89-5	3	31	0.097	5	+	NR
2. Methyl cyclohexanecarboxylate	3568	4630-82-4	2	2	0.006	1	+	NR
3. Ethyl cyclohexanecarboxylate	3544	3289-28-9	0.5	0.5	0.002	1	+	NR
4. Cyclohexaneethyl acetate	2348	5452-75-5	2[b]	< 0.01[5]	0.006[9]	25	NR	NR
5. Cyclohexaneacetic acid	2347	5292-21-7	4	< 0.01[5]	0.013[7]	2	NR	NR
6. Ethyl cyclohexanepropionate	2431	10094-36-7	46[6]	< 0.01[5]	0.15[9]	25	NR	NR

NR = not reported

Table B2. Summary of metabolism and toxicity data for primary alcohols, aldehydes, carboxylic acids and related esters: unsubstituted monocyclics

Substance	FEMA No.	CAS No.	Metabolic data in vivo/ in vitro[10]	Oral LD$_{50}$ (mg/kg)	Repeated dose study NOAEL (mg/kg/day) (duration)[11]	Repeated dose study NOAEL (mg/kg/day) for a structurally related substance (duration)[11]
1. Cyclohexanecarboxylic acid	3531	98-89-5	+ [a,b,c,d]/ −	3265[e]	NR	125 (27 wk)[12f]
2. Methyl cyclohexanecarboxylate	3568	4630-82-4	− / −	3881[e]	NR	,,
3. Ethyl cyclohexanecarboxylate	3544	3289-28-9	− / −	3962[e]	NR	,,
4. Cyclohexaneethyl acetate	2348	5452-75-5	− / −	2190[g] 3200[h]	NR	,,
5. Cyclohexaneacetic acid	2347	5292-21-7	+ [i]/ −	NR	NR	,,
6. Ethyl cyclohexanepropionate	2431	10094-36-7	− / −	NR	NR	,,

NR = not reported

[a]Bernhard and Caflish-Weill, 1945; [b]Brewster et al., 1977a; [c]Brewster et al., 1977b; [d]Williams, 1959; [e]Moran et al., 1980; [f]Paynter, 1957; [g]Moreno, 1978; [h]Wohl, 1974; [i]Bernhard, 1937.

Table B3. Summary of usage data for primary alcohols, aldehydes, carboxylic acids and related esters: substituted monocyclics and polycyclics

Substance	FEMA No.	CAS No.	Annual volume 1982 (kg)	Annual volume 1987 (kg)	Most recent per capita intake (μg/kg/day)[1]	Approx. max use level (ppm)	Natural occurrence in food[2]	Consumption Ratio[3]
1. 2,2,3-Trimethylcyclo-pent-3-en-1-ylacetaldehyde	3592	4501-58-0	0.3	0.1	0.0003	10	+	NR
2. cis-5-Isopropenyl-cis-2-methylcyclopentan-1-carboxaldehyde	3645	55253-28-6	< 9[4]	< 0.01[5]	0.013[8]	15	NR	NR
3. Campholene acetate	3657	36789-59-0	< 23[4]	< 0.01[5]	< 0.073[8]	5	+	NR
4. α-Campholenic alcohol	3741	1901-38-8	907[4]	NR	3[8]	35	+	NR
5. p-Menth-1-en-9-al	3178	29548-14-9	0.2	< 0.01[5]	0.00003[7]	15	+ +	>10
6. 1-p-Menthen-9-yl acetate	3566	17916-91-5	0.5	< 0.01[5]	0.002[7]	10	+	NR
7. p-Menth-1,8-dien-7-al	3557	2111-75-3	NR	15	0.05	10	+ + +	10
8. p-Menth-1,8-dien-7-ol	2664	536-59-4	0.1	< 0.01[5]	0.00003[7]	10	+ + +	>100
9. p-Mentha-1,8-dien-7-yl acetate	3561	15111-96-3	5	0.5	0.002	20	+ +	2
10. 1,2,5,6-Tetrahydrocuminic acid	3731	71298-42-5	250[4]	NR	0.8[8]	200	+	NR
11. 2,6,6-Trimethylcyclohexa-1,3-dienyl methanal	3389	116-26-7	NR	0.3	0.001	10	+	NR
12. 2,6,6-Trimethyl-1-cyclohexen-1-acetaldehyde	3474	472-66-2	3	4	0.012	10	NR	NR
13. 2,6,6-Trimethyl-1&2-cyclohexen-1-carboxaldehyde	3639	432-25-7	0.1	NR	0.0003	0.5	+	>100
14. 2-Formyl-6,6-dimethylbicyclo(3.1.1.)hept-2-ene (myrtenal)	3395	564-94-3	9	150	0.5	2	+ + +	NR
15. Myrtenol	3439	515-00-4	0.1	0.1	0.0003	10	+ +	>100
16. Myrtenyl acetate	3765	1079-01-2	44[4]	< 0.01[5]	0.14[8]	35	+ +	NR
17. 2-Hydroxy-6,6-dimethylbicyclo[3.1.1]hept-2-enyl formate	3405	72928-52-0	< 0.01[5]	8	0.025	3	+ +	NR
18. Santalol (α&β)	3006	77-42-9	0.2	0.5	0.002	10	+	NR
19. Santalyl acetate	3007	1323-00-8	0.1	0.5	0.002	10	NR	NR

NR = not reported

Table B4. Summary of metabolism and toxicity data for primary alcohols, aldehydes, carboxylic acids and related esters: substituted monocyclics and polycyclics

Substance	FEMA No.	CAS No.	Metabolic data *in vivo*/ *in vitro*[10]	Oral LD$_{50}$ (mg/kg)	Repeated dose study NOAEL (mg/kg/day) (duration)[11]	Repeated dose study NOAEL (mg/kg/day) for a structurally related substance (duration)[11]
1. 2,2,3-Trimethylcyclo-pent-3-en-1-ylacetaldehyde	3592	4501-58-0	— / —	4300[a]	11.9[a] (90 days) 12	NA
2. *cis*-5-Isopropenyl-*cis*-2-methylcyclopentan-1-carboxaldehyde	3645	55253-28-6	— / —	3000[b]	NR	11.9[a] (90 days)[12]
3. Campholene acetate	3657	36789-59-0	— / —	3900[c]	NR	11.9[a] (90 days)[12]
4. α-Campholenic alcohol	3741	1901-38-8	— / —	1000–2000 ml/kg[d]	NR	11.9a (90 days)[12]
5. *p*-Menth-1-en-9-al	3178	29548-14-9	— / —	NR	NR	11.9[a] (90 days)[12]
6. 1-*p*-Menthen-9-yl acetate	3566	17916-91-5	— / —	NR	NR	750 (20 wk)[12w]
7. *p*-Menth-1,8-dien-7-al	3557	2111-75-3	+ / —	1720[e]	NR	11.9[a] (90 days)[12]
8. *p*-Menth-1,8-dien-7-ol	2664	536-59-4	+ / —	2100[f]	1280[g] (15 wk)	NA
9. *p*-Mentha-1,8-dien-7-ylacetate	3561	15111-96-3	— / —	NR	NR	1280 (15 wk) 750 (20 wk)[12w]
10. 1,2,5,6-Tetrahydrocuminic acid	3731	71298-42-5	— / + [h]	> 2500[i]	NR	11.9[a] (90 days)[12]
11. 2,6,6-Trimethylcyclohexa-1,3-dienyl methanal	3389	116-26-7	— / —	3500[j]; 5700[k]	NR	11.9[a] (90 days)[12]
12. 2,6,6-Trimethyl-1-cyclohexen-1-acetaldehyde	3474	472-66-2	— / —	NR	NR	11.9[a] (90 days)[12]
13. 2,6,6-Trimethyl-1-&2-cyclohexen-1-carboxaldehyde	3639	432-25-7	+ [l] / —	4000[m]	NR 1280[g] (15 wk)	11.9[a] (90 days)[12]
14. 2-Formyl-6,6-dimethylbicyclo[3.1.1]hept-2-ene (myrtenal)	3395	564-94-3	+ [n] / —	1800–2900[o]	NR 1280[g] (15 wk)	11.9[a] (90 days)[12]
15. Myrtenol	3439	515-00-4	+ [p] / —	NR	NR	1280[g] (15 wk)
16. Myrtenyl acetate	3765	1079-01-2	— / —	2600[q]; < 2500[r]	NR	1280[g] (15 wk)
17. 2-Hydroxy-6,6-dimethylbicyclo[3.1.1]hept-2-enyl formate	3405	72928-52-0	— / —	> 5000[s]	NR	1280[g] (15 wk)
18. Santalol (α&β)	3006	77-42-9	+ [t] / —	3800[u]	15[v] (13 wk)	1280[g] (15 wk)
19. Santalyl acetate	3007	1323-00-8	— / —	> 5000[u]	NR	1280[g] (15 wk)

NA = not applicable   NR = not reported

[a]BIBRA, 1976; [b]Pellmont, 1973a; [c]Borriston Research Laboratories, Inc, 1979; [d]Levenstein, 1982; [e]Moreno, 1978; [f]Moreno, 1977; [g]Haag and Gould, 1994; [h]Brewster *et al.*, 1977a; [i]Levenstein, 1981; [j]Pellmont, 1967; [k]Pellmont, 1969; [l]Williams, 1959; [m]Pellmont, 1973b; [n]Ishida *et al.*, 1989; [o]Moreno, 1982; [p]Eriksson and Levin, 1990; [q]Moreno, 1982; [r]Kynoch, 1977; [s]Moreno, 1972; [t]Hildebrandt, 1902; [u]Keating, 1972; [v]Gaunt *et al.*, 1971; [w]Hagan *et al.*, 1967.

Table B5. Summary of usage data for ketones, secondary alcohols and related esters. Functional group on the ring: unsubstituted monocyclics

Substance	FEMA No.	CAS No.	Annual volume 1982 (kg)	Annual volume 1987 (kg)	Most recent per capita intake (μg/kg/day)[1]	Approx. max use level (ppm)	Natural occurrence in food[2]	Consumption Ratio[3]
1. Cyclohexyl formate	2353	4351-54-6	0.3	3	0.0009	35	NR	NR
2. Cyclohexyl acetate	2349	622-45-7	903	255	0.81	105	+	NR
3. Cyclohexyl propionate	2354	6222-35-1	15[6]	< 0.01[5]	0.048[9]	30	NR	NR
4. Cyclohexyl butyrate	2351	1551-44-6	18	2	0.006	45	+	NR
5. Cyclohexyl iso-valerate	2355	7774-44-9	0.5	0.3	0.001	55	NR	NR

NR = not reported

Table B6. Summary of metabolism and toxicity data for ketones, secondary alcohols and related esters. Functional group on the ring: unsubstituted monocyclics

Substance	FEMA No.	CAS No.	Metabolic data in vivo/ in vitro[10]	Oral LD$_{50}$ (mg/kg)	Repeated dose study NOAEL (mg/kg/day) (duration)[11]	Repeated dose study NOAEL (mg/kg/day) for a structurally related substance (duration)[11]
Cyclohexanol	NA	108-93-0	+ [a]/ −	2060[b], 2600[c]	400[d] (13 wk)[12,13] 50[e] (10 wk)[14]	NA
1. Cyclohexyl formate	2353	4351-54-6	− / −	NR	NR	400 (13 wk)[12,13]; 50[e] (10 wk)[14]
2. Cyclohexyl acetate	2349	622-45-7	− / −	> 5000[f], 6600[g]	NR	,,
3. Cyclohexyl propionate	2354	6222-35-1	− / −	NR	NR	,,
4. Cyclohexyl butyrate	2351	1551-44-6	− / −	> 5000[h]	NR	,,
5. Cyclohexyl iso-valerate	2355	7774-44-9	− / −	NR	NR	,,

NA = not applicable   NR = not reported

[a]Elliott et al., 1943; Pohl, 1925; Sasaki, 1917; Treon et al., 1943; [b]Bar and Griepentrog, 1967; [c]Treon et al., 1943; [d]Perbellini et al., 1981; [e]Treon et al., 1943; [f]Moreno, 1977; [g]Carpenter, 1974; [h]Moreno, 1982.

Table B7. Summary of usage data for ketones, secondary alcohols and related esters. Functional group on the ring: substituted monocyclics cyclopentanone and cyclohexanone derivatives

Substance	FEMA No.	CAS No.	Annual volume 1982 (kg)	Annual volume 1987 (kg)	Most recent *per capita* intake (µg/kg/day)[1]	Approx. max use level (ppm)	Natural occurrence in food[2]	Consumption Ratio[3]
1. 1-Methyl-1-cyclopenten-3-one	3435	2758-18-1	NR	0.14[6]	0.0004[9]	5	+	NR
2. 2-Hexylidenecyclopentanone	2573	17373-89-6	0.1	< 0.01[5]	0.0006[7]	30	NR	NR
3. iso-Jasmone	3552	11050-62-7	0.5	3	0.010	10	NR	NR
4. 3-Methyl-2-(2-pentenyl)-2-cyclopenten-1-one	3196	488-10-8	40	50	0.2	10	+	> 10
5. 3-Methyl-2-cyclohexen-1-one	3360	1193-18-6	NR	0.1	0.0003	0.5	+	NR
6. 3-Methyl-5-propyl-2-cyclohexen-1-one	3577	3720-16-9	< 0.01[5]	2	0.006	15	NR	NR
7. 2,2,6-Trimethylcyclohexanone	3473	2408-37-9	0.1	0.3	0.001	5	+	NR
8. Isophorone	3553	78-59-1	2	2	0.006	5	+	> 10
9. 2-sec-Butylcyclohexanone	3261	14765-30-1	20	8	0.03	150, 1100*	NR	NR
10. Tetramethylethylcyclohexenone	3061	999999-25-9	8	2	0.006	30	NR	NR

NR = not reported

*In chewing gum.

Table B8. Summary of metabolism and toxicity data for ketones, secondary alcohols and related esters. Functional group on the ring: substituted monocyclics cyclopentanone and cyclohexanone derivatives

Substance	FEMA No.	CAS No.	Metabolic data in vivo/in vitro[10]	Oral LD50 (mg/kg)	Repeated dose study NOAEL (mg/kg/day) (duration)[11]	Repeated dose study NOAEL (mg/kg/day) for a structurally related substance (duration)[11]
1. 1-Methyl-1-cyclopenten-3-one	3435	2758-18-1	− / +[a]	NR	NR	250 (103 wk)
2. 2-Hexylidenecyclopentanone	2573	17373-89-6	− / +[a]	> 5000[b]	2.9[c] (13 wk)[12]	NA
3. iso-Jasmone	3552	11050-62-7	− / −	> 5000[d]	NR	2.9 (13 wk)[12]
4. 3-Methyl-2-(2-pentenyl)-2-cyclopenten-1-one	3196	488-10-8	− / −	NR	NR	250 (103 wk)
5. 3-Methyl-2-cyclohexen-1-one	3360	1193-18-6	− / +[a]	NR	NR	250 (103 wk)
6. 3-Methyl-5-propyl-2-cyclohexen-1-one	3577	3720-16-9	− / −	5000[e]	NR	250 (103 wk)
7. 2,2,6-Trimethylcyclohexanone	3473	2408-37-9	− / −	NR	NR	370 (13 wk) 250 (103 wk)
8. Isophorone	3553	78-59-1	+[f] / +[a]	1870–3160[g]	500[h] (13 wk); 250[h] (103 wk)	NA
9. 2-sec-Butylcyclohexanone	3261	14765-30-1	− / −	2300[i], 2400[j]	370[k] (13 wk)	NA
10. Tetramethylethylcyclohexenone	3061	999999-25-9	− / −	NR	40.51[l] (13 wk)	NA

NR = not reported   NA = not applicable

[a]Portoghese *et al.*, 1989; [b]Moreno, 1980; [c]Posternak *et al.*, 1969; [d]Moreno, 1974; [e]Moreno, 1982; [f]Dutertre-Catella *et al.*, 1978; Truhaut *et al.*, 1970; [g]Coquet, 1977; Exxon, 1982a; Smyth *et al.*, 1970; Union Carbide, 1975; [h]Bucher *et al.*, 1986; [i]Givaudan-Roure, 1965; [j]Moreno, 1978; [k]Hummler, 1969; [l]Research Laboratories, Inc, Firmenich & Cie, 1963.

Table B9. Summary of usage data for ketones, secondary alcohols and related esters. Functional group on the ring: substituted monocyclics carvone derivatives

Substance	FEMA No.	CAS No.	Annual volume 1982 (kg)	Annual volume 1987 (kg)	Most recent per capita intake (μg/kg/day)[1]	Approx. max use level (ppm)	Natural occurrence in food[2]	Consumption Ratio[3]
1. p-Menthan-2-one	3176	499-70-7	0.2	3	0.010	30	+	NR
2. p-Menthan-2-ol	3562	499-69-4	37[6]	< 0.01[5]	0.15[9]	15	+	NR
3. p-Menth-8-en-2-one	3565	7764-50-3	< 0.01[5]	921	3	20	+ + +	4
4. Dihydrocarveol	2379	619-01-2	10	320	1	200	+ +	NR
5. Dihydrocarvyl acetate	2380	20777-49-5	2	0.3	0.001	20	+ +	> 1000
6. Carvone	2249	99-49-0	20,700	52,300	170	350	+ + +	2
7. Carveol	2247	99-48-9	100	744	2.4	30	+ +	> 3
8. Carvyl acetate	2250	97-42-7	90	190	0.6	65	+	> 10
9. Carvyl propionate	2251	97-45-0	0.5	0.2	0.0006	55	N R	NR

NR = not reported

Table B10. Summary of metabolism and toxicity data for ketones, secondary alcohols and related esters. Functional group on the ring: substituted monocyclics carvone derivatives

Substance	FEMA No.	CAS No.	Metabolic data in vivo/in vitro[10]	Oral LD$_{50}$ (mg/kg)	Repeated dose study NOAEL (mg/kg/day) (duration)[11]	Repeated dose study NOAEL (mg/kg/day) for a structurally related substance (duration)[11]
1. p-Menthan-2-one	3176	499-70-7	− / −	NR	NR	600 (103 wk)[12a] 750 (103 wk)[12b]
2. p-Menthan-2-ol	3562	499-69-4	− / −	NR	NR	600 (103 wk)[12a]
3. p-Menth-8-en-2-one (dihydrocarvone)	3565	7764-50-3	− / −	> 5000[c]	NR	125 (16 wk)[d] 750 (103 wk)[12b]
4. Dihydrocarveol	2379	619-01-2	+ [e]/ −	> 5000[f]	NR	600 (103 wk)[12a]
5. Dihydrocarvyl acetate	2380	20777-49-5	− / −	> 5000[g]	NR	600 (103 wk)[12a]
6 Carvone	2249	99-49-0	+ [h]/ −	766–3710[i]	125 (1 year)[12d] 750 (103 wk)[12b]	NA
7. Carveol	2247	99-48-9	+ [e]/ −	3000[k]	NR	600 (103 wk)[12a] 750 (103 wk)[12b]
8. Carvyl acetate	2250	97-42-7	− / −	> 5000[l]	NR	600 (103 wk)[12a] 750 (103 wk)[12b]
9. Carvyl propionate	2251	97-45-0	− / −	> 5000[l]	NR	600 (103 wk)[12a] 750 (103 wk)[12b]

NR = not reported   NA = not applicable

[a]NCI, 1978; [b]NTP, 1990; [c]Moreno, 1977; [d]Hagan et al., 1967; [e]Hamalainen, 1912; [f]Moreno, 1977; [g]Moreno, 1980; [h]Ishida et al., 1989; Williams, 1959; [i]Jenner et al., 1964; Levenstein, 1976; [j]Fischer and Beilig, 1940; [k]Keating, 1972; [l]Levenstein, 1976.

Table B11. Summary of usage data for ketones, secondary alcohols and related esters. Functional group on the ring: substituted monocyclics menthone derivatives

Substance	FEMA No.	CAS No.	Annual volume 1982 (kg)	Annual volume 1987 (kg)	Most recent *per capita* intake (μg/kg/day)[1]	Approx. max use level (ppm)	Natural occurrence in food[2]	Consumption Ratio[3]
1. Menthol	2665	89-78-1	44,400	54,400	17	2300	+ + +	4
2. *d-neo*-Menthol	2666	20752-34-5	0.1	140	0.44	595	+	NR
3. Menthone	2667	89-80-5	7530	13,300	42	70	+ + +	8
4. *dl-iso*-Menthone	3460	491-07-6	19	0.5	0.002	600	+	> 10,000
5. Menthyl acetate	2668	16409-45-3	2180	2940	9	55	+	5
6. Menthyl *iso*-valerate	2669	16409-46-4	140	0.3	0.001	25	+	> 1000
7. *l*-Menthyl lactate	3748	59259-38-0	NR	0.5	0.002	800	NR	NR
8. *p*-Menth-1-en-3-ol	3179	491-04-3	0.1	< 0.01[5]	0.00037	15	+	> 10,000
9. *d*-Piperitone	2910	6091-50-5	5	50	0.16	40	+ + +	> 10
10. 3-l-Menthoxypropane-1,2-diol	3784	87061-04-9	2150[4]	NR	7[8]	4000	NR	NR

NR = not reported

Table B12. Summary of metabolism and toxicity data for ketones, secondary alcohols and related esters. Functional group on the ring: substituted monocyclics menthone derivatives

Substance	FEMA No.	CAS No.	Metabolic data *in vivo*/*in vitro*[10]	Oral LD$_{50}$ (mg/kg)	Repeated dose study NOAEL (mg/kg/day) (duration)[11]	Repeated dose study NOAEL (mg/kg/day) for a structurally related substance or component (duration)[11]
1. Menthol	2665	89-78-1	+ [a]/ + [b]	940–4380[c]	600 (103 wk)[12d]	NA
2. *d-neo*-Menthol	2666	20752-34-5	+ [e]/ −	4000[f]	NR	600 (103 wk)[12d]
3. Menthone	2667	89-80-5	+ [g]/ −	1600–1950[h]	NR	,,
4. *dl-iso*-Menthone	3460	491-07-6	+ [i]/ −	NR	NR	,,
5. Menthyl acetate	2668	16409-45-3	− / −	> 5000, 7000[j]	NR	,,,
6. Menthyl *iso*-valerate	2669	16409-46-4	− / −	> 5000[k]	NR	
						2500 (90 days) Ac[12k]; 600 (103 wk) Alc[12d]
7. *l*-Menthyl lactate	3748	59259-38-0	− / −	6350–7870[m]	NR	600 (103 wk)[12d]
8. *p*-Menth-1-en-3-ol	3179	491-04-3	− / −	NR	NR	,,
9. *d*-Piperitone	2910	6091-50-5	− / −	NR	NR	600 (103 wk)[12d]; 750 (103 wk)[12n]
10. 3-l-Menthoxypropane-1,2-diol	3784	87061-04-9	− / −	5600, 5800[o]	NR	600 (103 wk)[12d]

NA = not applicable   NR = not reported   Ac = acid   Alc = alcohol

[a]Atzl *et al.*, 1972; Deichmann and Thomas, 1943; Eisenburg *et al.*, 1955; Kaffenberger and Doyle, 1990; Madyastha and Srivatsan, 1988a; Quick, 1924; Somerville *et al.*, 1984; White *et al.*, 1987; Williams, 1938 and 1939; Yamaguchi *et al.*, 1994; [b]Madyastha and Srivatsan, 1988a; FAO, 1967; [c]FDA, 1975; Jenner *et al.*, 1964; Wokes, 1932; [d]FDA, 1993; [e]Williams, 1940; [f]Wokes, 1932; [g]Neubauer, 1901; Williams, 1940; [h]Igimi and Ide, 1974; Levenstein, 1973; [i]Williams, 1940; [j]Levenstein, 1973; Shelanski and Moldovan, 1972; [k]Moreno, 1976; [l]Amoore *et al.*, 1978; [m]FDRL, 1984; LPT, 1977; [n]NTP, 1990; [o]FEMA, 1988.

Table B13. Summary of usage data for ketones, secondary alcohols and related esters. Functional group on the ring: substituted monocyclics *iso*-pulegone derivatives

Substance	FEMA No.	CAS No.	Annual volume 1982 (kg)	Annual volume 1987 (kg)	Most recent *per capita* intake (μg/kg/day)[1]	Approx. max use level (ppm)	Natural occurrence in food[2]	Consumption Ratio[3]
1. Isopulegone	2964	29606-79-9	10	< 0.01[5]	0.03[7]	15	+	NR
2. Isopulegol	2962	89-79-2	40	1370	4.3	20	+ + +	NR
3. Isopulegol acetate	2965	57576-09-7	50	21	0.07	10	NR	NR
4. Pulegone	2963	89-82-7	15	15	0.05[7]	35	+ + +	> 100
5. *p*-Mentha-1,4(8)-dien-3-one	3560	491-09-8	15[6]	< 0.01[5]	0.00048[9]	5	+ +	< 10

NR = not reported

Table B14. Summary of metabolism and toxicity data for ketones, secondary alcohols and related esters. Functional group on the ring: substituted monocyclics *iso*-pulegone derivatives

Substance	FEMA No.	CAS No.	Metabolic data *in vivo*/ *in vitro*[10]	Oral LD_{50} (mg/kg)	Repeated dose study NOAEL (mg/kg/day) (duration)[11]	Repeated dose study NOAEL (mg/kg/day) for a structurally related substance (duration)[11]
1. Isopulegone	2964	29606-79-9	− / + [d]	NR	NR	20[b] (28 days)
2. Isopulegol	2962	89-79-2	+ [a,j]/ + [l]	1200[c]	NR	600[e] (103 wk)
3. Isopulegol acetate	2965	57576-09-7	− / −	> 5000[c]	NR	600[e] (103 wk)
4. Pulegone	2963	89-82-7	+ [f,g,h,i]/ + [j]	470[a]	20[b] (28 days)	NA
5. *p*-Mentha-1,4(8)-dien-3-one	3560	491-09-8	+ [k]/ −	NR	NR	20[b] (28 days)

NR = not reported   NA = not applicable

[a]Moreno, 1975; [b]Thorup *et al.*, 1983; [c]Lynch, 1971; [d]Madyastha and Raj, 1990; [e]NCI, 1978; [f]Thomassen *et al.*, 1991; [g]Madyastha and Raj, 1993; [h]Nelson *et al.*, 1992; [i]Moorthy *et al.*, 1989; [j]Thomassen *et al.*, 1990; [k]Gordon *et al.*, 1982; [l]Boutin *et al.*, 1985.

Table B15. Summary of usage data for ketones, secondary alcohols and related esters. Functional group on the ring: bicyclic and macrocyclic substances

Substance	FEMA No.	CAS No.	Annual volume 1982 (kg)	Annual volume 1987 (kg)	Most recent per capita intake (μg/kg/day)[1]	Approx. max use level (ppm)	Natural occurrence in food[2]	Consumption Ratio[3]
1. *d*-Camphor	2230	464-49-3	1910	907	3	25	+ + +	4
2. *l*-Borneol	2157	507-70-0	60	90	0.3	10	+ + +	10
3. Bornyl formate	2161	7492-41-3	0.5	< 0.01[5]	0.002[7]	20	NR	NR
4. Bornyl acetate	2159	76-49-3	80	4	0.013	15	+ + +	> 100
5. Bornyl valerate	2164	7549-41-9	28[6]	< 0.01[5]	0.09[9]	20	NR	NR
6. Bornyl *iso*-valerate	2165	76-50-6	3	< 0.01[5]	0.010[7]	20	+	NR
7. *iso*-Borneol	2158	124-76-5	80	< 0.01[5]	0.3[7]	30	+ +	NR
8. *iso*-Bornyl formate	2162	1200-67-5	0.5	2	0.006	5	NR	NR
9. *iso*-Bornyl acetate	2160	125-12-2	310	210	0.7	15	+	NR
10. *iso*-Bornyl propionate	2163	2756-56-1	0.5	< 0.01[5]	0.002[7]	10	+	NR
11. *iso*-Bornyl *iso*-valerate	2166	7779-73-9	25	0.5	0.002	20	NR	NR
12. *d*-Fenchone	2479	4695-62-9	18	3	0.010	15	+ + +	NR
13. Fenchyl alcohol	2480	1632-73-1	70	90	0.3	10	+ + +	10
14. 1,3,3-Trimethyl-2-norbonyl acetate	3390	13851-11-1	0.1	< 0.01[5]	0.0003[7]	0.5	+	NR
15. Verbenol	3594	473-67-6	2[6]	< 0.01[5]	0.006[9]	1	+	NR
16. 2(10)-Pinen-3-ol	3587	5947-36-4	2[6]	< 0.01[5]	0.006[9]	1	+	NR
17. 4,4a,5,6,7,8-Hexahydro-4,4a-dimethyl-3(2*H*)naphthalenone (nootkatone)	3166	4674-50-4	44	50	0.2	20	+ +	> 10
18. Dihydronootkatone	3776	20489-53-6	122	< 0.01[5]	0.04[8]	5	+	NR
19. 7-Methyl-4,4a,5,6-tetrahydro-2(3*H*)-naphthalenone	3715	34545-88-5	NR	350	1.1	2	NR	NR
20. 3-Methyl-1-cyclopentadecanone	3434	541-91-3	0.2	0.1	0.0003	0.05	NR	NR
21. Cycloheptadeca-9-en-1-one	3425	542-46-1	0.1	0.3	0.001	0.05	NR	NR

NR = not reported

Table B16. Summary of metabolism and toxicity data for ketones, secondary alcohols and related esters. Functional group on the ring: bicyclic and macrocyclic substances

Substance	FEMA No.	CAS No.	Metabolic data in vivo/ in vitro[10]	Oral LD$_{50}$ (mg/kg)	Repeated dose study NOAEL (mg/kg/day) (duration)[11]	Repeated dose study NOAEL (mg/kg/day) for a structurally related substance (duration)[11]
1. *d*-Camphor	2230	464-49-3	+ [a,b,c]/ + [c]	> 5000[d]	NR	800[16]
						15 (13 wk)[r]; 75 (8 wk)[dd,13]
2. *l*-Borneol	2157	507-70-0	+ [e,f,g,h,i]/ + [j]	6500[k]	526 (31 days)[bb]	15 (13 wk)[r]
3. Bornyl formate	2161	7492-41-3	− / −	NR	NR	,,
4. Bornyl acetate	2159	75-49-3	+ [l]/ −	> 5000[m]	NR	,,
5. Bornyl valerate	2164	7549-41-9	− / −	NR	NR	,,
6. Bornyl *iso*-valerate	2165	76-50-6	− / −	> 5000[n]	NR	,,
7. *iso*-Borneol	2158	124-76-5	− / −	5200[o]	NR	,,
8. *iso*-Bornyl formate	2162	1200-67-5	− / −	> 5000[p]	NR	,,
9. *iso*-Bornyl acetate	2160	125-12-2	− / −	> 10,000[q]	15 (13 wk)[r]	NA
10. *iso*-Bornyl propionate	2163	2756-56-1	− / −	> 5000[s]	NR	15 (13 wk)[r]
11. *iso*-Bornyl- *iso*-valerate	2166	7779-73-9	− / −	NR	NR	,,
12. *d*-Fenchone	2479	4695-62-9	+ [t]/ −	6160[u]	420 (16 days)[v]	,,
13. Fenchyl alcohol	2480	1632-73-1	+ [w]/ −	> 5000[d]	NR	,,
14. 1,3,3-Trimethyl-2-norbonyl acetate	3390	13851-11-1	− / −	> 5000[x]	NR	,,
15. Verbenol	3594	473-67-6	+ [y,z]/ + [aa]	NR	NR	375, 600 (2 years)[cc]
16. 2(10)-Pinen-3-ol	3587	5947-36-4	+ [z]/ −	NR	NR	,,
17. 4,4a,5,6,7,8-Hexahydro-4,4a-dimethyl-3(2*H*)naphthalenone (nootkatone)	3166	4674-50-4	+ [ee]/ −	> 5000[ff]	NR	125, 750 (2 years)[cc]; 3.37 (13 wk)[kk]
18. Dihydronootkatone	3776	20489-53-6	− / −	> 5000[gg]	NR	,,
19. 7-Methyl-4,4a,5,6-tetrahydro-2(3*H*)-naphthalenone	3715	34545-88-5	− / −	1220[hh]	NR	,,
20. 3-Methyl-1-cyclopentadecanone	3434	541-91-3	− / −	> 5000[ii]	NR	,,
21. Cycloheptadeca-9-en-1-one	3425	542-46-1	− / −	> 5000[jj]	NR	,,

NR = not reported   NA = not applicable

[a]Koppel *et al.*, 1982; [b]Robertson and Hussain, 1969; [c]Leibman and Ortiz, 1973; [d]Moreno, 1976; [e]Quick, 1927; [f]Miller *et al.*, 1933; [g]Pryde and Williams, 1934; [h]Fishman, 1940; [i]Wagreich *et al.*, 1941; [j]Iliroi *et al.*, 1995; [k]Moreno, 1972; [l]Williams, 1959; [m]Owen, 1971; [n]Denine, 1973; [o]Moreno, 1977; [p]Levenstein, 1975; [q](by gavage) Fogleman and Margolin, 1970; [r]Gaunt *et al.*, 1971; [s]Moreno, 1973; [t]Reinartz and Zanke, 1936; [u](by gavage) Jenner *et al.*, 1964; [v]Rimini, 1901; [w]Hamalainen, 1912; [x]Moreno, 1975; [y]Erikkson and Levin, 1990; [z]Ishida *et al.*, 1981; [aa]White and Agosin, 1980; [bb]Miller *et al.*, 1933; [cc]NTP, 1990; [dd]Skramlik, 1959; [ee]Asakawa *et al.*, 1986; [ff]Moreno, 1977; [gg]Sedlacek, 1985; [hh]Mallory *et al.*, 1982; [ii]Moreno, 1977; [jj]Moreno, 1974; [kk]Posternak *et al.*, 1969.

Table B17. Summary of usage data for ketones, secondary alcohols and related esters. Functional group on the side chain

Substance	FEMA No.	CAS No.	Annual volume 1982 (kg)	Annual volume 1987 (kg)	Most recent *per capita* intake (μg/kg/day)[1]	Approx. max use level (ppm)	Natural occurrence in food[2]	Consumption Ratio[3]
1. 4-(2,6,6-Trimethyl-cyclohex-1-enyl)but-2-en-4-one (β-damascone)	3243	23726-92-3	60	50	0.16	10	+	NR
2. α-Damascone	3659	43052-87-5	< 0.01[5]	2	0.006	5	+	NR
3. δ-Damascone	3622	57378-68-4	0.5	3	0.01	5	NR	NR
4. 4-(2,6,6-Trimethyl-cyclohexa-1,3-dienyl)but-2-en-4-one	3420	23696-85-7	20	24	0.076	5	+	> 5
5. 1,4-Dimethyl-4-acetyl-1-cyclohexene	3449	43219-68-7	0.4	< 0.01[5]	0.001[7]	5	+	NR
6. α-Ionone	2594	127-41-3	1970	767	2.4	60	+	> 1
7. β-Ionone	2595	14901-07-6	1470	549	1.7	275	+	> 10
8. γ-Ionone	3175	79-76-5	80	< 0.01[5]	0.25[7]	20	+	> 1
9. α-Ionol	3624	25312-34-9	0.3	< 0.01[5]	0.001[7]	5	+	> 1000
10. β-Ionol	3625	22029-76-1	0.3	0.5	0.002	5	+	> 1000
11. Dihydro-α-ionone	3628	31499-72-6	0.1	0.1	0.0003	5	+	> 1000
12. Dihydro-β-ionone	3626	17283-81-7	0.2	< 0.01[5]	0.0006[7]	10	+	> 10
13. Dihydro-β-ionol	3627	3293-47-8	0.1	< 0.01[5]	0.0003[7]	15	NR	NR
14. Dehydrodihydroionone	3447	20483-36-7	0.4	< 0.01[5]	0.0013[7]	10	NR	NR
15. Dehydrodihydroionol	3446	57069-86-0	46[4]	< 0.01[5]	0.00015[8]	10	NR	NR
16. Methyl-α-ionone	2711	127-42-4	110	35	0.11	50	NR	NR
17. Methyl-β-ionone	2712	127-43-5	35	0.9	0.003	50	NR	NR
18. Methyl-δ-ionone	2713	7784-98-7	70	6	0.019	25	NR	NR
19. Allyl-α-ionone	2033	79-78-7	38	130	0.41	10	NR	NR
20. α-Irone	2597	79-69-6	12	15	0.048	5	+	NR
21. α-*iso*-Methylionone	2714	127-51-5	5	5	0.016	35	NR	NR

NR = not reported

Table B18. Summary of metabolism and toxicity data for ketones, secondary alcohols and related esters. Functional group on the side-chain

Substance	FEMA No.	CAS No.	Metabolic data in vivo/ in vitro[10]	Oral LD$_{50}$ (mg/kg)	Repeated dose study NOAEL (mg/kg/day) (duration)[11]	Repeated dose study NOAEL (mg/kg/day) for a structurally related substance (duration)[11]
1. 4-[(2,6,6)-Trimethyl-cyclohex-1-enyl]but-2-en-4-one (β-damascone)	3243	23726-92-3	− / +[k]	2200[j] 2920[m] > 2000[n]	2.35 (13 wk)[m,12]	NR
2. α-Damascone	3659	43052-87-5	− / −	1800[a]	NR	2.35 (13 wk)[m]
3. δ-Damascone	3622	57378-68-4	− / −	1820[r]	NR	2.35 (13 wk)[m,12]
4. 4-[(2,6,6)-Trimethyl-cyclohexa-1,3-dienyl)]but-2-en-4-one	3420	23696-85-7	− / +[k]	> 2000[s]	NR	2.35 (13 wk)[m,12]
5. 1,4-Dimethyl-4-acetyl-1-cyclohexene	3449	43219-68-7	− / −	NR	NR	2.35 (13 wk)[m]
6. α-Ionone	2594	127-41-3	+[b] / −	4590*[e]	10 (≥ 90 days)* 11 (12 wk)[g]	NR
7. β-Ionone	2595	14901-07-6	+[a] / −	4590*[q]	10 (≥ 90 days)* 11 (12 wk)[g]	NR
8. γ-Ionone	3175	79-76-5	− / −	NR	NR	10 (≥ 90 days)*[d]
9. α-Ionol	3624	25312-34-9	− / −	7400[c]	NR	10 (≥ 90 days)*[d]
10. β-Ionol	3625	22029-76-1	− / −	5700[p] > 1220; < 5000[p]	NR	10 (≥ 90 days)*[d]
11. Dihydro-α-ionone	3628	31499-72-6	− / −	> 5000[t]	NR	10 (≥ 90 days)*[d]
12. Dihydro-β-ionone	3626	17283-81-7	− / −	5700[v]	NR	10 (≥ 90 days)*[d]
13. Dihydro-β-ionol	3627	3293-47-8	− / −	7400[u]	NR	10 (≥ 90 days)*[d]
14. Dehydrodihydroionone	3447	20483-36-7	− / −	NR	NR	10 (≥ 90 days)*[d]
15. Dehydrodihydroionol	3446	57069-86-0	− / −	NR	NR	10 (≥ 90 days)*[d]
16. Methyl-α-ionone	2711	127-42-4	− / −	> 5000†[w]	NR	10 (≥ 90 days)*[d]
17. Methyl-β-ionone	2712	127-43-5	− / −	NR	NR	10 (≥ 90 days)*[d]
18. Methyl-δ-ionone	2713	7784-98-7	− / −	> 5000[w]	NR	10 (≥ 90 days)*[d]
19. Allyl-α-ionone	2033	79-78-7	− / −	8800[j]	NR	10 (≥ 90 days)*[d]
20. α-Irone	2597	79-69-6	− / −	> 5000[f]	5.2 (12 wk)[g,12]	NR
21. α-iso-Methylionone	2714	127-51-5	− / −	> 5000†[h]	3.55 (12 wk)[i,12]	NR

NR = not reported

*Data reported is for mixture consisting of α-ionone and β-ionone.
†Data reported is for mixture of methyl-α-ionone and α-iso-methylionone.
[a]Piccirillo et al., 1979; [b]Bielig and Hayasida, 1940; Ide and Toki, 1970; Parke and Rahman, 1969; Prelog and Meier, 1950; Prelog and Würsch, 1951; [c]Pellmont, 1978; [d]BIBRA, 1983; [e]Jenner et al., 1964; [f]Shelanski and Moldovan, 1972; [g]Bar and Griepentrog, 1967; Oser et al., 1965; [h]Moreno, 1973; [i]Oser et al., 1965; [j]Leberco Labs, Inc, 1982; [k]Portoghese et al., 1989; [l]Firmenich, Inc, 1969; [m]Posternak and Vodoz, 1975; [n]Firmenich, Inc, 1986; [o]Pellmont, 1977; [p]Moreno, 1980; [q]Jenner et al., 1964; [r]Moran et al., 1980; [s]Firmenich, Inc, 1986; [t]Moreno, 1976; [u]Pellmont, 1977; [v]Pellmont, 1977; [w]Moreno, 1973.

Table B19. Summary of usage data for tertiary alcohols and related esters

Substance	FEMA No.	CAS No.	Annual volume 1982 (kg)	Annual volume 1987 (kg)	Most recent per capita intake (μg/kg/day)[1]	Approx. max use level (ppm)	Natural occurrence in food[2]	Consumption Ratio[3]
1. α-Terpineol	3045	98-55-5	13,100	5530	18	85	+ + +	4
2. Terpinyl formate	3052	2153-26-6	10	< 0.01[5]	0.03[7]	15	+	NR
3. Terpinyl acetate	3047	80-26-2	2020	2030	6	30	+ + +	1/30
4. Terpinyl propionate	3053	80-27-3	70	5	0.016	15	+	NR
5. Terpinyl butyrate	3049	2153-28-8	9	33	0.1	15	+	NR
6. Terpinyl *iso*-butyrate	3050	7774-65-4	0.1	< 0.01[5]	0.0003[7]	15	NR	NR
7. Terpinyl *iso*-valerate	3054	1142-85-4	5[6]	< 0.01[5]	< 0.016[9]	15	NR	NR
8. *p*-Menth-3-en-1-ol	3563	586-82-3	2[6]	< 0.01[5]	0.0063[9]	50	+	2
9. *p*-Menth-8-en-1-ol (β-terpineol)	3564	138-87-4	320[6]	< 0.01[5]	1.0[9]	50	+ + +	> 5
10. 4-Carvomenthenol	2248	562-74-3	160	270	0.85	41	+ + +	> 100
11. 4-Thujanol	3239	546-79-2	0.2	< 0.01[5]	0.00063[7]	16	+ +	> 1000
12. 2-Ethyl-1,3,3-trimethyl-2-norbornanol	3491	18368-91-7	0.3	0.1	0.00032	0.5	NR	NR
13. Methyl 1-acetoxycyclohexyl ketone	3701	52789-73-8	22[8]	< 0.01[5]	0.070[4]	0.5	NR	NR

NR = not reported

Table B20. Summary of metabolism and toxicity data for tertiary alcohols and related esters

Substance	FEMA No.	CAS No.	Metabolic data in vivo/in vitro[10] substance (duration)[11]	Oral LD$_{50}$	Repeated dose study NOAEL (mg/kg/day) (mg/kg)	Repeated dose study NOAEL (mg/kg/day) for a structurally related (duration)[11]
1. α-Terpineol	3045	98-55-5	+ [a,b]/ −	4300*[c]	NR	500 (20 wk)[12d]
2. Terpinyl formate	3052	2153-26-6	− / −	> 5000[e]	NR	,,
3. Terpinyl acetate	3047	80-26-2	− / −	5075[f]	500 (20 wk)[12d]	NA
4. Terpinyl propionate	3053	80-27-3	− / −	> 5000[g]	NR	500 (20 wk)[12d]
5. Terpinyl butyrate	3049	2153-28-8	− / −	NR	NR	,,
6. Terpinyl *iso*-butyrate	3050	7774-65-4	− / −	> 5000[h]	NR	,,
7. Terpinyl *iso*-valerate	3054	1142-85-4	− / −	NR	NR	,,
8. *p*-Menth-3-en-1-ol	3563	586-82-3	− / −	NR	NR	,,
9. *p*-Menth-8-en-1-ol (β-terpineol)	3564	138-87-4	− / −	4300*[i]	NR	,,
10. 4-Carvomenthenol	2248	562-74-3	− / −	1300[k]	NR	500 (20 wk)[12d]
11. 4-Thujanol	3239	546-79-2	− / −	NR	NR	,,
12. 2-Ethyl-1,3,3-trimethyl-2-norbornanol	3491	18368-91-7	− / −	NR	NR	15 (13 wk)[j]
13. Methyl 1-acetoxycyclohexyl ketone	3701	52789-73-8	− / −	2150[l]	NR	,,

NR = not reported   NA = not applicable

*LD$_{50}$ reported for a mixture of α- and β-terpineol.

[a]Madyastha and Srivatsan, 1988b; [b]Horning *et al.*, 1976; [c]Moreno, 1971a; [d]Hagan *et al.*, 1967; [e]Moreno, 1976; [f]Jenner *et al.*, 1964; [g]Moreno, 1973; [h]Moreno, 1982; [i]Moreno, 1971b; [j]Gaunt *et al.*, 1971; [k]Moreno, 1977; [l]Borriston Research Laboratories, Inc, 1980.

APPENDIX B TABLE ENDNOTES

1. The *per capita* intake is based on the annual volume reported in the most recent industry survey (NAS, 1987). For substances in which the annual volume was reported to be less than 0.01 kg, the *per capita* intake is calculated using a previously reported annual volume either from a prior NAS survey (e.g. 1970 or 1982) or the manufacturer (i.e. the anticipated annual volume, see footnote 2). The calculation includes the assumptions that (1) only 60% of the flavour volume was reported in the NAS survey and, therefore, the total annual volume was 1.67 times greater than reported, and (2) only 10% of the population, the "eaters only", consumed the entire volume.

2. Substances occurring in more than 10, six to 10 and one to five foods are given the respective designations + + +, + + and +.

3. Consumption ratios were calculated using the method by Stofberg and Kirschman (1985) and data by Stofberg and Grundschober (1987).

4. The volume cited is the anticipated annual volume, which was the maximum amount of flavour estimated to be used annually by the manufacturer at the time the material was proposed for flavour use. Subsequent national surveys (NAS/NRC Surveys, 1982 and 1987) revealed no reported use of the substance as a flavour ingredient. Therefore, the anticipated volume is probably a gross exaggeration of the annual volume. The *per capita* intake is calculated based on the assumptions that (1) only 60% of the flavour volume was reported by the manufacturer and, therefore, the total annual volume was 1.67 times greater than reported, and (2) only 10% of the population, the "eaters only", consumed the entire volume.

5. Respondents to the NAS Survey were requested to report annual poundages of less than 0.1 lb to two digits past the decimal point.

6. Annual volume from 1970 NAS Survey since less than 0.01 lb were reported in 1982 and 1987.

7. Calculated using 1982 NAS Survey annual volume.

8. Calculated using the anticipated annual volume.

9. Calculated using 1970 NAS Survey annual volume.

10. A "+" indicates that hydrolysis data have been reported for the specific flavour ingredient. A "−" indicates that hydrolysis data for the specific flavour ingredient have not been reported and hydrolysis is presumed based on data from structurally related substances.

11. The NOAEL is reported for oral administration of the substance in either food or drinking water, unless noted.

12. The study was performed at a single dose level or multiple dose levels that produced no adverse effects and, therefore, a NOAEL was not determined. The NOAEL is probably higher than the reported dose level that produced no adverse effects.

13. The test substance was administered as a component of a mixture.

14. Dose was calculated (Beliles and Schulz, 1993) from results of an inhalation study.

15. Dose was administered by intraperitoneal injection.

16. Reported in a reproduction study.

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