EVALUATION OF THE HEALTH ASPECTS OF OIL OF CLOVES
AS A FOOD INGREDIENT

APRIL 1973

Prepared for

Bureau of Foods
Food and Drug Administration
Department of Health, Education, and Welfare
Washington, D. C.

Contract No. FDA 72-85
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Life Sciences Research Office
Federation of American Societies
for Experimental Biology
9650 Rockville Pike
Bethesda, Maryland 20014
NOTICE

This report is one of a series of evaluations of the health aspects of the Generally Recognized as Safe (GRAS) food substances that are being made by the Life Sciences Research Office (LSRO) of the Federation of American Societies for Experimental Biology (FASEB) under contract with the Food and Drug Administration (FDA) of the U.S. Department of Health, Education, and Welfare. The Federation recognizes that the safety of GRAS substances is of national significance, and its resources are particularly suited to marshalling the opinions of knowledgeable scientists to assist in these evaluations. The Life Sciences Research Office, established in 1962 to make scientific assessments in the biomedical sciences, is conducting these studies.

Qualified scientists were selected as consultants to make a continuing review, analysis, and evaluation of the available information on each of the GRAS substances. These scientists, designated the Select Committee on GRAS Substances, were chosen for their competence and judgment with due consideration for balance and breadth in the appropriate professional disciplines. Members of the Select Committee on GRAS Substances who have contributed to this report are named in Section VII. The Select Committee's evaluations are being made independently of FDA or any other governmental or nongovernmental group.

These reports are approved by the Select Committee prior to submission to FDA. Although most LSRO consultants are members of FASEB constituent societies, the reports do not necessarily reflect the views of the Federation as a corporate body or carry the endorsement of the members of its constituent societies.

C. JeHeff Carr, Ph.D., Director
Life Sciences Research Office
FASEB
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I. INTRODUCTION

Under terms of FDA Contract 72-85, FASEB's Life Sciences Research Office was requested to evaluate the health aspects of using oil of cloves as a food ingredient, primarily on the basis of information contained in a monograph furnished by FDA (1), summarizing the world's scientific literature from 1920 through 1970, and in certain supplemental documents available as of April 1973. Oil of cloves is a food substance that has been generally recognized as safe (GRAS) under the provisions of Section 121.101 of the Code of Federal Regulations (21 CFR 121.101, revised January 1, 1972).

As indicated in the Food, Drug, and Cosmetic Act [21 USC 321 (s)], GRAS substances are exempt from the requirement of the pre-marketing clearance for food additives. It is stated in 21 CFR 121.1 that GRAS means general recognition of safety by experts qualified by scientific training and experience to evaluate the safety of substances on the basis of scientific data derived from published literature. This section of the Code also indicates that expert judgment is to be based on the evaluation of results of credible toxicological testing or for those substances used in food prior to January 1, 1958, on a reasoned judgment founded in experience with common food use, and is to take into account reasonably anticipated patterns of consumption, cumulative effects in the diet, and safety factors appropriate for the utilization of animal experimentation data. It is recognized further (21 CFR 121.3) that it is impossible to provide assurance that any substance is absolutely safe for human consumption.

The Select Committee on GRAS Substances of LSRO is making its evaluations of these substances in full recognition of the foregoing provisions. In reaching its conclusions on safety the Select Committee, in accord with FDA's guidelines, is relying primarily on the absence of substantive evidence of or reasonable grounds to suspect a significant risk to the public health, and realizes that a decision, based on such reasoned judgment, is expected even in instances where the available information is qualitatively or quantitatively limited. The Committee is also aware that biological testing, like all of science, is dynamic. Accordingly, the Committee's decisions, based as they are on the information now available, cannot anticipate and be guided by experiments not yet done or by the results of tests that may be reconduted, using new technologies that are constantly being evolved. These
decisions will need to be reviewed as new or better information becomes available.

In this context, the LSRO Select Committee on GRAS Substances has reviewed the available information on oil of cloves and submits its interpretation and assessment in this report, which is intended for the use of FDA in determining the future status of oil of cloves under the Federal Food, Drug and Cosmetic Act.

II. BACKGROUND INFORMATION

Cloves are the dried, unopened flower buds and twig tips of the clove tree, *Eugenia caryophyllata* Thunberg, native to tropical Asia. The bulk of present commercial production comes from Tanzania and the Malagasy Republic, with the latter currently supplying most of United States imports. The stage of development of the flower bud at time of harvest is critical to high quality of the product. Clusters of buds and stems are harvested by hand, sun dried, graded, and marketed. High grade dried buds are usually marketed as whole cloves. The lower quality buds, stems, and leaves, including those obtained during the regular practice of topping the trees, are usually used in production of the essential oils (1, 2).

Clove bud oil, clove stem oil, and clove leaf oil are the essential oils obtained by steam distillation of these products. Yield of oil depends on the quality of the material distilled, precautions exercised, and efficiency of the still. Yields amount to about 18 percent for clove buds, about 5 percent for stems, and about 2 percent for leaves. Two other commercial products are used in foods: clove bud extract, obtained by solvent extraction of clove buds, and clove bud oleoresin which contains triglycerides and other lipids in addition to the essential oil.

There are differences in composition of the three essential oils (2). Clove bud oil contains 70 to 90 percent or more eugenol, 2 to 17 percent eugenyl acetate, 5 to 12 percent α and β caryophyllene, and traces of caryophyllene epoxide, methyl salicylate, methyl-n-amyl ketone, methyl-n-heptyl ketone, methyl-n-amyl carbinol, methyl-n-heptyl carbinol, methyl alcohol, methyl benzoate, furfural, alpha methyl furfural, furfuryl alcohol, vanillin, and possibly β pinene, valeraldehyde, methyl furfuryl alcohol, and a dimethyl furfural.
Clove stem and clove leaf oils have been investigated less intensively than has clove bud oil. In general, the stem and leaf essential oils contain a higher proportion of free eugenol, making them a preferred source of eugenol for subsequent conversion to iso-eugenol, derivatives of eugenol and iso-eugenol, and vanillin. The stem and leaf oils contain little or no eugenyl acetate. Caryophyllene, furfural, and methyl alcohol have been identified in the stem oil, but many of the compounds, present in traces in the bud oil and associated with its characteristic fruity odor, are absent or present in much smaller quantities in the stem and leaf oils. Unlike the bud oil, the stem and leaf oils appear to contain traces of naphthalene and a bicyclic sesquiterpene alcohol.

The Food Chemicals Codex (3) provides specifications for the bud, stem, and leaf oils of food grade as follows:

Clove bud oil, not less than 85 percent, by volume, of phenols as eugenol, angular rotation between \(-1^\circ\) 30' and 0', refractive index between 1.527 and 1.535 at 20', specific gravity between 1.038 and 1.060, and not more than 3 ppm arsenic, 40 ppm heavy metals as lead, or 10 ppm of lead;

Clove stem oil, not less than 89 percent and not more than 95 percent, by volume, of phenols as eugenol, angular rotation between \(-1.5^\circ\) and 0', refractive index between 1.534 and 1.538 at 20', specific gravity between 1.048 and 1.056;

Clove leaf oil, not less than 84 percent and not more than 88 percent, by volume, of phenols as eugenol, angular rotation between \(-2^\circ\) and 0', refractive index between 1.531 and 1.535 at 20', specific gravity between 1.036 and 1.046.

All of the oils should be stored in tight glass, aluminum, tin-lined, or stainless steel containers protected from light and heat.

Cloves were used in China before the time of Christ and were known in Western Europe as early as the sixth century. They have been used in the U.S. since colonial times. While the principal use of cloves and clove oil today is in the flavoring of foods, they are also used to flavor tobacco and are important as analgesics and germicides (2, 4). Eugenol is also utilized for similar medicinal purposes,
although it is slightly less active as an antiseptic than the natural oil. In addition, it has been used in the treatment of gastric or duodenal ulcers and has considerable dental value as a disinfectant for root-canals, a local anodyne for relief of hypersensitive dentine and inflamed vital pulps, and a component of temporary fillings for carious teeth (4).

Cloves, the essential oils of clove buds, stems, and leaves as well as eugenol, isoeugenol, and several derivatives of both of these phenols are used to impart clove or clove-like flavor to the foods listed and in the amounts indicated in Table 1 (5).

Of the substances indicated in the column headings of Table 1, cloves, clove bud extract, clove bud oil, clove bud oleoresin, clove leaf oil, clove stem oil, and eugenol are on both the FDA GRAS list (6) and the FEMA GRAS list (7). Isoeugenol, the listed derivatives of eugenol and isoeugenol, and beta caryophyllene are on the FEMA GRAS list only, together with several of the trace constituents of clove bud oil indicated earlier in this section of this report.

The United States is reported to be the world's third largest consumer of clove products (2). Importations of cloves and clove oil show no trend during the period 1965 through 1971, amounting to an average of about 2.5 million pounds of cloves and stems (grouped together in import statistics) and about 1.2 million pounds of clove oil annually (8). The Select Committee has no information to indicate whether the clove and clove product content of the food categories listed in Table 1 has changed significantly in recent years.

III. CONSUMER EXPOSURE DATA

A National Research Council subcommittee (5) has provided information on the possible daily human intake of cloves and products derived from them in the total diet, as shown in Table 2 for individuals in various age groups. The Select Committee has converted these figures to possible intake per kilogram of body weight.

These intake figures must be considered in respect to the total use and importations of cloves and the products derived from them. The NRC subcommittee has pointed out that its calculations of intakes
<table>
<thead>
<tr>
<th></th>
<th>Cloves</th>
<th>Clove bud extract</th>
<th>Clove bud oil</th>
<th>Clove bud oleoresin</th>
<th>Clove leaf oil</th>
<th>Clove stem oil</th>
<th>Eugenol, isoeugenol &amp; derivatives¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baked goods &amp; baking mixes</td>
<td>385.1240²</td>
<td>110.130</td>
<td>40.90</td>
<td>240.280</td>
<td>40.100</td>
<td>30.40</td>
<td>150.300</td>
</tr>
<tr>
<td>Fats &amp; oils</td>
<td>185.340</td>
<td>-</td>
<td>20.85</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cheese</td>
<td>15.15</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Frozen dairy desserts &amp; mixes</td>
<td>20.40</td>
<td>25.40</td>
<td>35.40</td>
<td>160.175</td>
<td>20.25</td>
<td>110.130</td>
<td>60.80</td>
</tr>
<tr>
<td>Processed fruits, juices &amp; drinks</td>
<td>190.330</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Meat products</td>
<td>300.970</td>
<td>165.220</td>
<td>130.160</td>
<td>240.305</td>
<td>320.460</td>
<td>430.525</td>
<td>25.30</td>
</tr>
<tr>
<td>Processed vegetables &amp; juices</td>
<td>485.485</td>
<td>-</td>
<td>10.20</td>
<td>-</td>
<td>10.10</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Condiments, relishes</td>
<td>1015.2360</td>
<td>130.180</td>
<td>60.90</td>
<td>130.150</td>
<td>20.45</td>
<td>15.60</td>
<td>60.120</td>
</tr>
<tr>
<td>Soft candy</td>
<td>220.225</td>
<td>80.100</td>
<td>100.140</td>
<td>625.650</td>
<td>60.100</td>
<td>390.415</td>
<td>95.130</td>
</tr>
<tr>
<td>Sugar &amp; confections</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>625.750</td>
</tr>
<tr>
<td>Gelatins, puddings, fillings</td>
<td>-</td>
<td>-</td>
<td>315.350</td>
<td>25.30</td>
<td>5.10</td>
<td>100.125</td>
<td>65.85</td>
</tr>
<tr>
<td>Soups &amp; soup mixes</td>
<td>150.800</td>
<td>-</td>
<td>1.10</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Snack foods</td>
<td>-</td>
<td>-</td>
<td>160.320</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Nonalcoholic beverages</td>
<td>130.140</td>
<td>10.15</td>
<td>10.15</td>
<td>130.150</td>
<td>10.10</td>
<td>45.50</td>
<td>30.45</td>
</tr>
<tr>
<td>Alcoholic beverages</td>
<td>1.3</td>
<td>125.150</td>
<td>150.200</td>
<td>725.775</td>
<td>85.105</td>
<td>500.600</td>
<td>&lt;1.1</td>
</tr>
<tr>
<td>Gravies, sauces</td>
<td>30.130</td>
<td>-</td>
<td>10.255</td>
<td>265.525</td>
<td>260.270</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Hard candy</td>
<td>-</td>
<td>-</td>
<td>20.160</td>
<td>-</td>
<td>65.65</td>
<td>205.1070</td>
<td>40.55</td>
</tr>
<tr>
<td>Chewing gum</td>
<td>-</td>
<td>-</td>
<td>190.2850</td>
<td>-</td>
<td>285.855</td>
<td>140.190</td>
<td>105.265</td>
</tr>
</tbody>
</table>

¹Includes: total of eugenol, isoeugenol, eugenyl acetate, isoeugenyl acetate, eugenyl benzoate, isoeugenyl ethyl ether, eugenyl formate, isoeugenyl formate, eugenyl methyl ether, isoeugenyl methyl ether, and isoeugenyl phenyl acetate

²First figure is usual content; second maximum content in ppm.
in most cases are overstated, often by considerable margins.* That human intakes are undoubtedly overstated in Table 2 is borne out by the following calculations.

The NRC subcommittee has also provided data (5) to show that the use of cloves for food purposes in the U.S., adjusted to 100 percent, was 865,558 pounds (393,436 kg), in 1970. On the basis of a U.S. population of 200 million, the per capita per day average intake would be only 5.4 mg rather than the 172.75 mg shown in Table 2. Using the annual use figures for total oils, extracts, and oleoresin, the corresponding figure is 1.7 mg rather than 274.73; for eugenol, 0.6 mg rather than 6.99.

On the basis of the average annual imports (8) of cloves and stems (grouped together in the import statistics) of 2.5 million pounds (1.14 million kg), and assuming all were used as such in food, the average daily per capita intake would not exceed 15.6 mg. Similarly, on the basis of average annual imports of clove oils of 1.2 million pounds (0.54 million kg) and assuming all were used in food, the average daily per capita intake would not exceed 7.4 mg.

Another approximate intake reference point can be derived from these data and from statistics on the synthetic production of eugenol (11). Since a maximum of 18 percent essential oil could be present in imported cloves and stems, the 2.5 million pounds imported could contain a maximum of 450,000 pounds (204,545 kg) of clove oil. This, added to the 1.2 million pounds of imported clove oil, totals 1.65 million pounds (750,000 kg) of oil containing a maximum of 90 percent of the major ingredient eugenol. Thus, the equivalent of 1.485 million pounds (675,000 kg) of imported eugenol could conceivably be available to add to U.S. foods. This, added to the 435,000 pounds (197,727 kg) of synthetic eugenol produced in 1970, and assuming all was used in food, would make possible a maximum average daily per capita intake of 12 mg of eugenol from all known sources.

*An explanation for such overstatements is detailed in Section XI, "Significance and Use of Data in Safety Evaluations," of the NRC subcommittee report (5). The Select Committee finds this explanation reasonable and concurs in the first recommendation of Section XII of the same report that "In order to conduct a more accurate survey on the intake of substances used in food processing, food consumption data collected specifically for this purpose are needed."
TABLE 2
CLOVES AND RELATED SUBSTANCES

Possible Daily Intake, Mg

<table>
<thead>
<tr>
<th>Total intake</th>
<th>Intake, mg per kg body weight*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Substances</td>
<td></td>
</tr>
<tr>
<td>0-5 mos.</td>
<td>6-11 mos.</td>
</tr>
<tr>
<td>Av</td>
<td>Max</td>
</tr>
<tr>
<td>3.70</td>
<td>8.43</td>
</tr>
<tr>
<td>1.25</td>
<td>2.14</td>
</tr>
<tr>
<td>0.42</td>
<td>1.07</td>
</tr>
<tr>
<td>1.95</td>
<td>4.57</td>
</tr>
<tr>
<td>0.59</td>
<td>1.47</td>
</tr>
<tr>
<td>1.08</td>
<td>3.06</td>
</tr>
<tr>
<td>0.12</td>
<td>0.32</td>
</tr>
<tr>
<td>0.06</td>
<td>0.12</td>
</tr>
<tr>
<td>0.10</td>
<td>0.19</td>
</tr>
<tr>
<td>0.07</td>
<td>0.13</td>
</tr>
<tr>
<td>0.68</td>
<td>0.12</td>
</tr>
<tr>
<td>0.14</td>
<td>0.26</td>
</tr>
<tr>
<td>0.04</td>
<td>0.07</td>
</tr>
<tr>
<td>0.04</td>
<td>0.07</td>
</tr>
<tr>
<td>0.16</td>
<td>0.30</td>
</tr>
<tr>
<td>0.06</td>
<td>0.10</td>
</tr>
<tr>
<td>0.01</td>
<td>0.03</td>
</tr>
</tbody>
</table>

*Calculations based on an average weight of 60 kg for an adult (9) and the following estimated weights of infants by age groups: 0-5 mos., 5 kg; 6-11 mos., 8 kg; 12-23 mos., 11 kg (10).
On the basis of these considerations, the Select Committee regards the figures given in Table 2 as levels that are highly unlikely to be achieved by any of the age groups, but more likely, are generous overestimates of the content of cloves and related products in the daily diet.

The Joint FAO/WHO Committee on Food Additives has established a conditional acceptable daily intake for eugenol of 5 mg per kg (12, 13). The term "conditional" means that the substance may be employed with an adequate margin of safety provided experts have reviewed the available evidence for the particular use.

IV. BIOLOGICAL STUDIES

Oral toxicity studies on cloves or the essential oils derived from them and from clove stems and leaves have not been reported but animal studies have been conducted on their major constituent eugenol.

Within 24 hours, 44 to 90 percent of the eugenol (1500 to 2500 mg) fed was absorbed, depending on the dose (14). It was detectable in the blood, lungs, kidneys, and liver of both rabbits and rats for up to several hours after ingestion and was excreted principally in the urine.

The oral LD₅₀ of eugenol for fasted rats has been reported to be 1930 mg per kg (15) and 2680 mg per kg (16, 17); for fasted mice 3000 mg per kg (16), for fasted guinea pigs 2150 mg per kg (16), and for the dog >200 mg per kg (18). An oral LD₅₀ of 500 mg per kg for the rat has also been reported (19).

Short-term (less than half of the life span) oral administration of 10 doses of 200 mg eugenol per kg over a three week period to dogs revealed no observable changes in their activity and behavior, suggesting no cumulative effects of the compound (18).

Daily oral doses of 895 mg eugenol per kg given to rats over four days produced mild liver lesions, with slight discoloration and blunting of the edges of the lobes (17). In another study, daily doses of increasing amounts of eugenol, beginning at 1400 mg per kg for 34 days, were orally administered to 20 male rats. Fifteen lived to receive the maximum 4000 mg per kg and eight survived the full term of the study. Pathological studies showed "coalescent areas" in the mucosa of the
forestomach with thick flaky white material and minute ulcers at these very high levels. Microscopically, there was a moderately severe degree of hyperkeratosis of the stratified squamous epithelium. A slight degree of osteoporosis was also noted in the bone (18). In a longer term study, at a level of 10,000 ppm of eugenol in the diet over a nineteen week period, no effect was noted in 10 male and 10 female weanling rats (20).

Five percent emulsions of clove oil or eugenol stimulate alkaline secretion of the gastric mucosa, often destroying the mucous barrier through desquamatory action (21). However, the mucosa was completely resurfaced with new squamous cells 36 hours after removal of the eugenol although additional time was needed to restore normal physiological function (22, 23, 24). In another study, eugenol was reported to inhibit glucosiduronic acid conjugation, indicating to the investigators, an interference with mucopolysaccharide formation in tissues with possible gastric ulcer formation (25). Intraperitoneally injected eugenol and isoeugenol were inactive in inhibiting mouse hepatic microsomal enzyme function in contrast to active compounds such as safrole (26). Both eugenol methyl ether and safrole as well as their isomers, fed to rats at a level of 10,000 ppm in the diet for 15 days, increased the activity of hepatic microsomal hydroxylating enzymes, with the methylenedioxy compounds being the more active (27). The significance of this enzyme enhancement is unknown.

Despite their irritating effect when applied to the shaved dorsal skin of mice, preliminary studies indicate that clove oil and eugenol were not active as tumor-inducing agents (28).

No studies have been reported on the mutagenicity, teratogenicity, or carcinogenicity of orally administered cloves or related substances. However, guaiacol, of which eugenol is the allyl derivative, administered subcutaneously in repeated doses of up to 0.4 cc of a 4 percent solution in olive oil, failed to produce tumors in rats in 17 to 19 months (29).

V. OPINION

The available information indicates that orally administered eugenol, the principal ingredient of cloves and products derived from them, is readily absorbed and excreted without accumulation. Eugenol exhibits a low degree of toxicity for the several animal species tested,
and only at levels far greater than those occurring in foods, does it appear to be able under some conditions to produce irritation of the squamous epithelium of the gastrointestinal tract. It is to be noted that cloves, clove oils and eugenol have long been used topically as analgesics, germicides, and for other purposes.

In the light of the foregoing, the Select Committee concludes that:

There is no evidence in the available information on cloves, clove oils and their principal constituent, eugenol, that demonstrates or suggests reasonable grounds to suspect a hazard to the public when they are used at levels that are now current or that might reasonably be expected in future.
VI. REFERENCES CITED


VII. SCIENTISTS CONTRIBUTING TO THIS REPORT

1. Members of the Select Committee on GRAS Substances:

Aaron M. Altschul, Ph.D., Professor, Department of Community Medicine and International Health, School of Medicine, Georgetown University, Washington, D. C.

Joseph F. Borzelleca, Ph.D., Professor of Pharmacology, Medical College of Virginia, Health Sciences Division, Virginia Commonwealth University, Richmond, Va.

Bert N. La Du, Jr., M.D., Ph.D., Professor and Chairman, Department of Pharmacology, New York University School of Medicine, New York, N. Y.

John R. McCoy, V.M.D., Professor of Comparative Pathology, New Jersey College of Medicine and Dentistry, Rutgers Medical School, New Brunswick, N. J.

Sanford A. Miller, Ph.D., Professor of Nutritional Biochemistry, Massachusetts Institute of Technology, Cambridge, Mass.

Gabriel L. Plaa, Ph.D., Professor and Chairman, Department of Pharmacology, University of Montreal Faculty of Medicine, Montreal, Canada.

Ralph G. H. Siu, Ph.D., Consultant, Washington, D. C.

John L. Wood, Ph.D., Distinguished Service Professor, Department of Biochemistry, University of Tennessee Medical Units, Memphis, Tenn.

George W. Irving, Jr., Ph.D. (Chairman), Research Associate, Life Sciences Research Office, Federation of American Societies for Experimental Biology, Bethesda, Md.
2. LSRO staff:

C. Jelleff Carr, Ph.D., Director, LSRO/FASEB.
Samuel B. Detwiler, Jr., Research Associate, LSRO/FASEB.
Kenneth D. Fisher, Ph.D., Research Associate, LSRO/FASEB.
Andrew F. Freeman, Research Associate, LSRO/FASEB.

Report submitted by:

September 14, 1973
Date

George W. Irving, Jr., Chairman
Select Committee on GRAS Substances