EVALUATION OF THE HEALTH ASPECTS OF MUSTARD
AND OIL OF MUSTARD AS FOOD INGREDIENTS

1975

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

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NOTICE

This report is one of a series of evaluations of the health aspects of the Generally Recognized as Safe (GRAS) or prior sanctioned food substances being made by the Federation of American Societies for Experimental Biology (FASEB) under contract no. 72-85 with the Food and Drug Administration (FDA), U.S. Department of Health, Education, and Welfare. The Federation recognizes that the safety of GRAS substances is of national significance, and that its resources are particularly suited to marshalling the opinions of knowledgeable scientists to assist in these evaluations. The Life Sciences Research Office (LSRO), established by FASEB in 1962 to make scientific assessments in the biomedical sciences, is conducting these studies.

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

Tentative reports are made available to the public for review in the Office of the Hearing Clerk, Food and Drug Administration, after announcement in the Federal Register, and opportunity is provided for any interested person to appear before the Select Committee at a public hearing to make oral presentation of data, information, and views on the substances covered by the report. The data, information, and views presented at the hearing are considered by the Select Committee in reaching its final conclusions. Reports are approved by the Select Committee and the Director of LSRO, and subsequently reviewed and approved by the LSRO Advisory Committee (which consists of representatives of each constituent society of FASEB) under authority delegated by the Executive Committee of the Federation Board. Upon completion of these review procedures the reports are approved and transmitted to FDA by the Executive Director of FASEB.

While this is a report of the Federation of American Societies for Experimental Biology, it does not necessarily reflect the opinion of all of the individual members of its constituent societies.

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

This report evaluates the health aspects of using mustard and oil of mustard as food ingredients. The evaluation has been based partly on the information contained in a scientific literature review (monograph) furnished by FDA (1), which summarizes the world's scientific literature from 1920 through 1970. * To assure completeness and currency as of the date of this report this information has been supplemented by searches of over 30 scientific and statistical reference sources and compendia that are generally recognized as available; use of new, relevant books and reviews and the literature citations contained in them; consideration of current literature citations obtained through computer retrieval systems of the National Library of Medicine; searches for relevant data in the files of FDA; and by the combined knowledge and experience of members of the Select Committee and the LSRO staff. In addition, announcement was made in the Federal Register of December 19, 1974 (39 FR 43865 and 43866) that opportunity would be provided for any interested person to appear before the Select Committee at a public hearing to make oral presentation of data, information, and views on the health aspects of using mustard and oil of mustard as food ingredients. The Select Committee received one request for such a hearing but this request was withdrawn.

As indicated in the Food, Drug and Cosmetic Act [21 USC 321(3)], GRAS substances are exempt from the premarketing clearance that is required for food additives. It is stated in 21 CFR 121.1, revised April 1, 1974 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. FDA recognizes 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 accordance with FDA's guidelines, is relying primarily on the absence of

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*The document is available from the National Technical Information Service, U.S. Department of Commerce, P.O. Box 1553, Springfield, Virginia 22161.

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substantive evidence of, or reasonable grounds to suspect, a significant risk to the public health, and realizes that a conclusion based on such reasoned judgment is expected even in instances where the available information is qualitatively or quantitatively limited. The Committee, aware that biological testing is dynamic, bases its conclusions on information now available; it cannot anticipate the results of experiments not yet conducted or those of tests that may be reconducted, using new technologies. These conclusions 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 mustard and oil of mustard and submits its interpretation and assessment in this report, which is intended for the use of FDA in determining the future status of these substances under the Federal Food, Drug, and Cosmetic Act.

II. BACKGROUND INFORMATION

The mustard used in food is derived primarily from the seed of certain plants of the family Cruciferae. _Brassica nigra_ (L.) Koch and _B. juncea_ (L.) Czerniaew are the principal sources of black mustard seed from which the products called brown mustard and oriental mustard are prepared. _B. nigra_ is cultivated chiefly in Italy and Holland; _B. juncea_ is cultivated in northern India and southern Russia. _B. alba_ (L.) Boiss or _Sinapis alba_ (L.) is the principal source of white mustard seed from which the product called yellow mustard is prepared. White mustard is a native annual of southern Europe but is now widely cultivated in many temperate countries, chiefly Canada (1-5).

Mustard is used by the food industry in several forms: whole seed; ground seed meal; mustard cake (ground mustard seed from which a portion of the fixed oil has been expressed); mustard flour (ground mustard cake with hulls removed); and prepared mustard. Mustard flour and prepared mustard appear to be the forms most frequently used. Both may contain mustard from more than one source and, particularly in prepared mustard, may also contain a number of additional materials. In mustard flour, for example, blends of two or more mustard flours from different sources, together with mill fractions which the mustard miller has available, are often used to impart subtle flavors. In prepared mustards, as another example, food specifications permit their preparation from ground seed of white and/or black mustard, with or without mustard flour or mustard cake, together with vinegar and with or without addition of sweetening agents, salt, and such spices as turmeric, ginger, and black pepper. Prepared mustards contain about 10 to 15 percent solids (5, 6).
The characteristic flavor of brown mustard (described as "nose heat" or "horseradish-like bite") is due to allyl isothiocyanate. This is present in the seed as part of the glucoside sinigrin, from which it is released, together with glucose and potassium bisulfate, by the action of the enzyme myrosinase which also is present in the seed. The allyl isothiocyanate thus released is sufficiently stable chemically to be readily steam distillable. The distilled product, known variously as oil of mustard, volatile oil of mustard, and natural allyl isothiocyanate, is the most commonly used commercial mustard oil. Yield of the oil from the seed ranges from 0.5 to more than 1.0 percent. As prepared, it consists of more than 90 percent allyl isothiocyanate; the remainder is chiefly allyl cyanide and carbon disulfide. However, most of the allyl isothiocyanate used in food is prepared synthetically. Allyl isothiocyanate volatilizes easily, is lost from open containers in four to six months, and is susceptible to decomposition in air and light (4, 5, 7).

The corresponding constituent of yellow mustard is p-hydroxybenzyl isothiocyanate. It is present in the seed as part of the glucoside sinalbin from which it is released, together with glucose and sinapine bisulfate, by the action of myrosinase. Sinalbin mustard oil is only sparingly volatile with steam but can be separated by solvent extraction. About 3 or 4 percent of p-hydroxybenzyl isothiocyanate is present in the defatted flour of white mustard seed (8, 9). When freshly prepared, sinalbin mustard oil is strongly pungent with a mildly burning taste, described as "mouth heat." However, its principal constituent, p-hydroxybenzyl isothiocyanate, is highly unstable, and hydrolyzes in a matter of hours, at room temperature, to p-hydroxybenzyl alcohol, di (p-hydroxybenzyl) disulfide, and p-hydroxybenzyl cyanide (4, 5, 7, 10-13). According to Bice (5) and Baróthy (14), these hydrolytic products do not contribute significantly to the flavor of prepared yellow mustard; added spices and other constituents of the seed such as sinapine (the choline ester of sinapic acid) are the chief sources of its flavor.

Some of the reactions referred to in the preceding paragraphs are given below:

\[
\text{Sinigrin} \xrightarrow{\text{myrosinase}} \text{CH}_2=\text{CH-CH}_2\text{-N}=\text{C}=\text{S} + \text{glucose} + \text{potassium bisulfate}
\]
\[
\text{allyl isothiocyanate}
\]

Under some conditions the following may also be formed:

\[
\text{CH}_2=\text{CH-CH}_2\text{-S-C≡N}
\]
\[
\text{allyl thiocyanate}
\]

\[
\text{CH}_2=\text{CH-CH}_2\text{-C≡N}
\]
\[
\text{allyl cyanide}
\]

\[
\text{S=C=S}
\]
\[
\text{carbon disulfide}
\]
It is specified in the Food Chemicals Codex that food grade "mustard oil, volatile (allyl isothiocyanate)," whether from natural or synthetic sources, should assay not less than 93 percent allyl isothiocyanate, and conform to stated ranges with respect to distillation temperature, refractive index and specific gravity. Maximum limits are specified for arsenic and heavy metals (15). The Codex contains no specifications for p-hydroxybenzyl isothiocyanate or other mustard products.

A number of other plant seeds, mainly Cruciferae, contain isothiocyanate oils that are referred to as mustard oils (7). There is no record of their commercial use, but the similarity of many of them to those described above has been established. For example, in rape seed, B. napus (L.), the characteristic ingredient of its volatile oil is allylcarbinyl isothiocyanate (16).

A comprehensive survey by a National Research Council (NRC) subcommittee (17) reports that brown and yellow mustard and allyl isothiocyanate are used as flavoring in food products as indicated in Table I.
Table I

Use of Mustard and Allyl Isothiocyanate in Foods

<table>
<thead>
<tr>
<th>Food category</th>
<th>Brown mustard</th>
<th>Yellow mustard</th>
<th>Allyl isothiocyanate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Usual</td>
<td>Max.</td>
<td>Usual</td>
</tr>
<tr>
<td></td>
<td>ppm</td>
<td>ppm</td>
<td>ppm</td>
</tr>
<tr>
<td>Condiments, relishes, salt substitutes</td>
<td>82,071</td>
<td>101,503</td>
<td>60,995</td>
</tr>
<tr>
<td>Fats and oils</td>
<td>9,838</td>
<td>18,344</td>
<td>3,470</td>
</tr>
<tr>
<td>Nuts, nut products</td>
<td>6,200</td>
<td>10,000</td>
<td></td>
</tr>
<tr>
<td>Gravies, sauces</td>
<td>4,859</td>
<td>5,170</td>
<td>235</td>
</tr>
<tr>
<td>Baked goods, baking mixes</td>
<td>3,000</td>
<td>3,000</td>
<td>590</td>
</tr>
<tr>
<td>Snack foods</td>
<td>820</td>
<td>820</td>
<td>13,506</td>
</tr>
<tr>
<td>Processed vegetables juices</td>
<td>179</td>
<td>216</td>
<td>1,345</td>
</tr>
<tr>
<td>Meat products</td>
<td>9</td>
<td>***</td>
<td>10,163</td>
</tr>
<tr>
<td>Fish products</td>
<td></td>
<td></td>
<td>1,000</td>
</tr>
<tr>
<td>Soups, soup mixes</td>
<td></td>
<td></td>
<td>30</td>
</tr>
<tr>
<td>Frozen dairy desserts, mixes</td>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Soft candy</td>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Gelatins, puddings, fillings</td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Beverages Type I (nonalcoholic)</td>
<td></td>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>

Blanks in the table mean that the substance is not added to the foods indicated; asterisks (*** ) in the table mean that (a) the substance is used in a processing phase of the foods indicated but residual levels in the food product are negligible or unknown, or (b) the substance is used in the foods indicated but usage levels were not provided by industry, or (c) the substance is in the foods indicated but the levels were considered to be reported incorrectly (see explanatory notes on exhibit 40 of reference 17).
Domestic production of mustard seed (color not specified) was 5,276,621 pounds (2,398,464 kg) in 1969; 60 percent was grown in California and Montana (18). Bureau of the Census statistics also show that imports of whole mustard seed (color not specified) increased about threefold between 1965 and 1972 to about 104 million pounds in 1972. Imports of ground and prepared mustard increased slightly less than two-fold over the same period, reaching just under 2 million pounds in 1972 (19). According to other estimates (5) about 57 million pounds of mustard seed (more than 75 percent white seed) were used by the U.S. food industry in 1972. The Select Committee has no information from which to determine if there has been significant change over the past decade in the mustard content of the food categories shown in Table I.

III. CONSUMER EXPOSURE DATA

The survey of the NRC subcommittee has provided information on the possible daily human intake of mustard in the diet (Table II) for various age groups (17). The Select Committee has converted these figures to possible intake per kilogram of body weight.

The NRC subcommittee has pointed out that its calculations of GRAS substance intakes in most cases are overstated, often by considerable margins.* The Select Committee believes that this is probably so in the case of mustard products. The NRC subcommittee has also provided data (17) to show that approximately the following amounts were used annually by the food industry in the United States in 1970: yellow mustard, 48.5 million pounds (22 million kg); brown mustard, 3.2 million pounds (1.45 million kg); allyl isothiocyanate, 33,000 pounds (15,000 kg). These figures have been derived from those given by the NRC subcommittee which they estimate to represent about 60 percent of actual use. Using a population of 210 million, these poundages would provide for an average per capita daily consumption of only 287,18, and 0.2 mg of yellow mustard, brown mustard, and allyl isothiocyanate, respectively, rather than the

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*An explanation for such overstatements is detailed in Section XI, "Significance and Use of Data in Safety Evaluations," of the NRC subcommittee's report (17). The Select Committee finds this explanation reasonable, and concurs in the first recommendation in 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 II

Possible Daily Intake of Mustard and Allyl Isothiocyanate

<table>
<thead>
<tr>
<th>Total intake, mg</th>
<th>Substance</th>
<th>Intake, mg per kg body weight&lt;sup&gt;3&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-5 mos.</td>
<td>6-11 mos.</td>
</tr>
<tr>
<td>0-5 mos.</td>
<td>Av</td>
<td>Max</td>
</tr>
<tr>
<td>17</td>
<td>47</td>
<td>324</td>
</tr>
<tr>
<td>16</td>
<td>30</td>
<td>204</td>
</tr>
<tr>
<td>&lt;1</td>
<td>&lt;1</td>
<td>1</td>
</tr>
<tr>
<td>Allyl isothiocyanate</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
</tr>
</tbody>
</table>

<sup>1</sup> Calculations based on an average weight of 60 kg for an adult (20), and the following estimated weights of infants by age groups: 0-5 mos., 5 kg; 6-11 mos., 8 kg; and 12-23 mos., 11 kg (21).

<sup>2</sup> Assumed to be flour prepared from white mustard seed; estimated to contain 1 to 2 percent mustard oil consisting chiefly of p-hydroxybenzyl isothiocyanate and/or its decomposition products.

<sup>3</sup> Assumed to be flour prepared from black or brown mustard seed; estimated to contain 1 to 2 percent mustard oil consisting chiefly of allyl isothiocyanate.
1622, 1396, and 6 mg given in Table II. The lower intake figures, based on poundage used, appear the more plausible since import and domestic production statistics show that some 90 million pounds of whole and ground mustard seed are available annually (18, 19). These statistics do not differentiate between white and brown mustard seed. However, in converting this 90 million pounds of whole and ground seed to the mustard flours used in food, loss of the fatty fraction would reduce the weight used to a figure closely approximating that indicated by the NRC subcommittee for total poundage of yellow and brown mustard reported to be used annually by the food industry (51.7 million pounds).

On the basis of these considerations, the Select Committee regards the figures in Table II as levels that are severalfold greater than those likely to be achieved by any of the age groups. It should also be noted in this connection that the isothiocyanates of brown and yellow mustard are either highly unstable or volatile, making the effective amount present in most foods as consumed unpredictably less than the amount added.

IV. BIOLOGICAL STUDIES

A. Brown mustard and its constituents

The oral LD₅₀ of allyl isothiocyanate from brown mustard seed for the rat is reported to be 339 mg per kg (22); the subcutaneous LD₅₀ for mice, 80 mg per kg (23). For rats, the oral LD₅₀ of allyl cyanide, reported to be a constituent of the essential oil of brown mustard, is 115 mg per kg (24); for rabbits and oral lethal dose of carbon disulfide, also reported to be a constituent of the essential oil of brown mustard, appears to be between 0.47 and 3.5 g (about 0.23 to 1.75 g per kg) (25).

Rats fed a diet containing allyl isothiocyanate (0.02 mg per kg body weight) for 70 days showed an increased level of fatty acid esters and cholesterol in the blood serum. Histologically, some fatty degeneration of the liver was noted but this was most marked when the allyl isothiocyanate was fed in combination with ethyl alcohol (15 ml of a 20 percent ethanol solution per kg animal weight) (26). Allyl isothiocyanate is reported to depress rate of growth slightly when fed to young rats at a level of 0.1 percent in the diet (estimated dose level, about 400 mg per kg) for 5 weeks; a more pronounced decrease in the growth rate occurred when the same amount of allyl isothiocyanate was administered by gavage rather than fed for an additional four weeks (27). In adult rats fed 2 mg of
allyl isothiocyanate per kg body weight it was observed that blood clotting
time was reduced, liver succinic dehydrogenase activity was increased,
and the activity of both xanthine oxidase and kidney D-amino acid oxidase
was decreased significantly when measured 4 hours after feeding (27).

Two groups of weanling Osborne-Mendel rats (5 males and 5
females in each group) received a daily dose (by stomach tube) of 20 mg
per kg and 50 mg per kg body weight of allyl isothiocyanate respectively,
for 20 days. Organ and tissue examination showed the following histo-
pathology: slight to moderate epithelial hyperplasia of the nonglandular
part of the stomach with acute to subacute ulcers from 2 to 6.5 mm
across in all animals at the higher dose and in half of the animals at the
lower dose; and minor inflammatory foci in the liver at the higher dose
level. However, the same authors reported that no effects were noted
after feeding weanling rats for 26 weeks on a diet containing up to 10,000
ppm (about 500 mg per kg per day) of allyl isothiocyanate (28). The
diet was prepared daily owing to the volatility of the compound. It is
noted that this dosage is significantly higher than the LD₅₀ reported from
the same laboratory (22); reasons for the variation are not clear.

The Select Committee was unable to find reports of long term
animal studies on brown mustard or its constituents, or of studies on the
absorption or excretion of these substances.

Teratologic evaluation was made of a sample of allyl oil of
mustard supplied by FDA and known to have been prepared from black
mustard seeds (B. nigra L.). The oral administration of up to 18.5 mg
per kg body weight daily to pregnant rats (6th through 15th days of gesta-
tion), up to 23.8 mg per kg to pregnant hamsters (6th through 10th days
of gestation), and up to 12.3 mg per kg to pregnant rabbits (6th through
18th days of gestation), had no clearly discernible effect on nidation or
on maternal or fetal survival (29). The number of abnormalities seen
in either soft or skeletal tissues of the test groups did not differ from
the number occurring spontaneously in the sham-treated controls. The
same result was obtained with pregnant mice at dosage levels up to 6.0
mg per kg (administered on the 6th through 15th days of gestation) but at
a level of 28.0 mg per kg there were indications (not statistically signifi-
cant) of increased fetal deaths and resorptions. These investigators
concluded that oil of mustard, at this high level may be fetotoxic in the
pregnant mouse without being teratogenic.

Studies of the toxicity and teratogenicity of mustard oil (allyl
isothiocyanate) in avian embryos revealed that it was not teratogenic under
the conditions employed (30). Doses up to 16 mg per kg were injected into
the air cell or the yolk of fertile eggs before incubation or after 96 hours
of incubation. The occurrence of abnormal embryos and those showing head, skeletal, visceral and limb abnormalities was not increased with the administration of mustard oil under any of these conditions. In the same series of experiments, mustard oil was found to be toxic to avian embryos when injected into the air cell of unincubated fertile eggs or after 96 hours of incubation, at a level of 0.6 mg per kg, and embryo mortality was increased with doses as low as 0.04 mg per kg when injected into the yolk.

Mutagenicity studies of mustard oil (allyl isothiocyanate) in which several assay procedures were employed, were uniformly negative (31). The assay procedures and the doses used were as follows: host-mediated assay using two strains of Saccharomyces cerevisiae at oral doses up to 130 mg of mustard oil per kg in mice; cytogenetic assay, in vivo, involving observation of aberrations in bone marrow metaphase chromosomes of rats administered up to 100 mg of mustard oil per kg orally; cytogenetic assay, in vitro, involving observation of aberrations in anaphase chromosomes of human embryonic lung cells in tissue culture exposed to levels up to 10 µg of mustard oil per ml; dominant lethal assay in rats using dosages of mustard oil up to 100 mg per kg.

No reports have been found on the testing of allyl cyanide or carbon disulfide for teratogenicity.

Allyl isothiocyanate, applied to the skin of female Sutter albino mice as a 50 percent solution in acetone with 2 percent corn oil twice weekly for 24 weeks, produced papillomas in 10 percent of the animals and carcinomas in 1 animal (32). A 0.003 normal solution of allyl isothiocyanate in acetone painted on the skin of mice twice weekly for 5 months produced no tumors (33). No skin tumors were found after 11 months in mice painted three times weekly with allyl isothiocyanate (34). No reports have been found concerning the potential carcinogenicity of orally administered allyl isothiocyanate.

Carbon disulfide in aqueous solution (concentration not reported) administered orally to dogs and rabbits, produced no tumors in three months (35). No reports have been found in which the carcinogenic potential of allyl cyanide has been studied by any route.

Based on the eating habits of a number of his patients who had experienced coronary attacks, Blair in 1965 (36) expressed the opinion that allyl isothiocyanate is the underlying cause of atheromatous sclerosis in a majority of cases. He has suggested that restriction of the "hot" condiments - e.g., mustard, pepper, and ginger - offers a means for prevention of essential hypertension and coronary disease. The editor of the Ohio State Medical Journal in which this article appeared, noted that the author's conclusions, while not substantiated, "represents a different viewpoint which may, or may not, be borne out in the experience of others."
B. Yellow mustard and its constituents

The subcutaneous LD_{so} of benzyl mustard oil (presumed to consist principally of p-hydroxybenzyl isothiocyanate from yellow mustard seed) for mice is stated to be 150 mg per kg (23). No data have been found on acute studies of orally administered benzyl mustard oil or its isothiocyanate. Acute toxicity studies, by any route of administration, of sinapine, p-hydroxybenzyl alcohol, di-(p-hydroxybenzyl) disulfide, p-hydroxybenzyl cyanide, or p-hydroxybenzyl amine, which are reported to occur in yellow mustard or to be present as decomposition products of p-hydroxybenzyl isothiocyanate have not been found in the literature.

The Select Committee was unable to find reports of short or long term studies on yellow mustard or its constituents, or studies on the absorption or excretion of these substances.

Only one experiment has been found that concerns the metabolic fate of mustard. In man, Perry et al. (37) fed 100 g of mustard to a male adult over a period of 1.5 hours and collected urine 24 hours after the start of consumption. Chromatographic analysis of the urine revealed the presence of p-hydroxybenzyl amine and an unidentified amine, metabolites that were not detectable in the urine when mustard was excluded from the diet. The mustard used was "prepared mustard, made from mustard seed, turmeric and other spices," but the source of the seed was not indicated. Since the allyl isothiocyanate of brown mustard could not conceivably give rise to a p-hydroxybenzyl metabolite, it can be reasoned that the mustard fed in this experiment was probably of the yellow variety and that the source of the p-hydroxybenzyl amine excreted could either be a separate constituent of white mustard seed as reported by Larsen (38) or could arise as a metabolite of p-hydroxybenzyl isothiocyanate; p-hydroxybenzyl amine has also been found in barley seeds (39).

No reports have been found on the carcinogenicity, teratogenicity, or mutagenicity of white mustard seed, or of its reported constituents, or of the degradation products of its constituents.

C. Mustard (variety unidentified)

The oral LD_{so} of the volatile oil of mustard (source not indicated) for the rat was reported to be 148 mg per kg; 50 mg per kg per day was tolerated by rats for eight weeks without noticeable injury (40).

For 30 days rats were fed a basal diet containing 10 percent mustard cake (source not indicated) supplemented with 20 percent of one of a number of vegetable oils including mustard oil (source not
indicated) (41). It is evident that the mustard cake used in the basal diet was mustard seed from which the fatty acid glycerides had been expressed, leaving some or all of the essential oil; and that the mustard oil used was the fatty acid glycerides of mustard seed containing some or all of the essential oil. Serum lipid levels decreased in animals receiving the mustard oil during the early part of the feeding, but subsequently the level showed a steady upward trend. Mustard oil also produced consistent and progressive increases in the liver lipid level. Similar effects were noted with some of the other vegetable oils tested, making it appear that the effects noted with mustard oil were not due to its essential oil constituents. No evidence of toxicity to the animals subjected to this regime was reported by the investigators (41).

In one study, the nutritive contribution of mustard oil (expressed) appeared to be limited to its fat content. The source of the mustard oil was not indicated. Feeding tests with rats showed that this mustard oil (assumed to be the expressed fatty acid glycerides of mustard seed, containing some or all of the essential oil) was entirely devoid of vitamin A activity, and tended to inactivate vitamin A when mixed with other foods. The tests also showed that, as a fat, mustard oil was as nutritious as other fats, provided adequate vitamin A intake was assured from other sources (42).

The possibility of synergism between mustard oil (source not indicated, but presumed from the context of the report to be expressed mustard oil containing the fatty acid glycerides as well as the essential oil) and cholesterol was studied by including 10 percent of mustard oil in the feed of growing chicks, with or without one percent cholesterol for eight weeks. Chicks on the diet containing mustard oil showed an increase in the level of nonesterified fatty acids in the blood plasma but no change in other plasma lipids. Chicks on diets containing both cholesterol and mustard oil showed an increase in total plasma cholesterol, β-lipoprotein cholesterol, triglycerides, and nonesterified fatty acids. The extent of hypercholesterolemia did not seem dependent on the degree of saturation of the mustard oil (43). A subsequent report from the laboratory of one of these investigators extended these observations to effects on portions of the aorta by feeding diets containing 10 percent mustard oil or other oils, with or without one percent cholesterol, to growing chicks for eight weeks (44). When such oils were fed without cholesterol, there was an increase in phospholipids in the aorta but no change in cholesterol; whereas cholesterol, with or without the oils, increased both cholesterol and phospholipids in the aorta. Further, the hexosamine content of the aorta decreased regardless of whether cholesterol was fed, implying a concomitant decrease in acid mucopolysaccharides. The hydroxyproline content of the aorta increased when both oils and cholesterol were fed, indicating that arterial collagen had increased. The latter effect seemed
to be proportional to the degree of hypercholesterolemia. Severity of changes were found to be unrelated to either the cholesterol level in the plasma or to the degree of unsaturation of the oils fed. In all respects, mustard oil behaved no differently than coconut, sesame, and hydrogenated peanut oils (44).

Observations were made of the short term physiological effects due to mustard (source not indicated), lemon, curry, paprika, and sugar fed as condiments in test diets of healthy young men. It was noted that increase in salivary flow was of about the same magnitude for all of these substances, while mustard and paprika were least effective in increasing salivary amylase activity (45).

Bandyopadhyay and Banerjee (46) fed up to 20 percent of various vegetable oils, including what is presumed to be expressed oil of mustard of unspecified source, with and without cholesterol, in the diet of rhesus monkeys for nine months. In the animals fed diets containing the mustard oil, diminished amounts of cholesterol deposits were found at autopsy in the thoracic and abdominal aortas, the brain, and the skin, while increased amounts of cholesterol were found in the liver. In a similar study, also with rhesus monkeys, Banerjee et al. (47) fed cholesterol and various vegetable oils (up to 20 percent of the diet) for six months. Periodic observations showed that the level of plasma cholesterol was raised and fecal cholesterol was reduced to the same degree, indicating that the vegetable oils probably facilitated intestinal absorption of cholesterol.

Twenty human adult volunteers were fed a breakfast containing 17 percent by weight (about one g per kg) of one of six different vegetable or animal fats, including mustard oil (source not identified but evidently the expressed oil containing the fatty acid glycerides as well as some or all of the essential oil). A blood sample, taken four hours after breakfast, was analyzed. The experiment was repeated on each of five successive days. Feeding mustard oil was found to have no effect on platelet content, serum cholesterol, Stypvan* time, or the fibrinolyzing property of the blood; whole blood clotting time was reduced as it was after feeding any of the fats, and fibrinogen content decreased, as it also did after feeding sesame oil; platelet adhesiveness increased as it also did after feeding groundnut, sesame or coconut oils (48).

Two mustard oils were tested for carcinogenic activity in XVII x C57 (black) hybrid mice by oral, cutaneous, subcutaneous, and intraperitoneal routes. There was no evidence of malignancy due to

*Stypvan: a preparation of Russell's viper venum, used as a thromboplastic agent in the one-stage prothrombin time test.
either oil at the site of treatment or in the remote organs even though
the animals were observed for their complete life span of over two
years (49). One of the oils was obtained from the Indian Agricultural
Research Institute, New Delhi, and the other from the Raj Oil Mills,
Bombay. Direct inquiry by the Select Committee of the authors and
the Indian Agricultural Research Institute provided little additional infor-
mation concerning the identity of these oils; the IARI sample was of
unknown origin and the Raj sample was a market sample and believed
to be a mixture. In more recent work the same investigators (50, 51)
found no evidence of carcinogenicity in mice administered laboratory
expressed refined mustard oil (seed source not indicated) by stomach
tube five days each week for their life span. Under the same conditions,
one of 27 mice fed a market sample of mustard oil (seed source not
indicated) developed transplantable gastric carcinoma in 13 months.

V. OPINION

The two mustards (brown and yellow) commonly used in the
United States are derived from two Brassica species that differ with
respect to the major chemical constituents of their essential oils.
The available information shows that the characteristic isothio-
cyanates, other known constituents, and decomposition products of the
essential oils of both mustards, have low orders of oral toxicity in
experimental animals. Further, there is no reported evidence that
orally administered doses of these constituents are carcinogenic, terato-
genic, or mutagenic. However, interpretation of the available
information suffers from a general lack of specificity on the part of
investigators concerning the identity of the particular mustard used in
their experiments, and from the fact that the major constituents of the
essential oils are either very volatile or unstable, making it specula-
tive how much of each of these substances was actually present in the
preparation tested. The industrial practice of using mixtures of mus-
tards to impart desired flavors compounds the problem of relating the
results of toxicological studies to conditions that may exist in foods.
For the same reasons, the amounts of the essential mustard oil con-
stituents actually present in foods at the time of consumption are unpre-
dictably variable, but must be less than the amounts calculated from the
reported daily human intake levels of mustard.

The Select Committee has made an exhaustive search for informa-
tion relevant to the health aspects of mustard and mustard oils as food
ingredients and is confident that the literature evaluated in this report is
representative and significant as a basis for judgment. However, because
of the long history of use of the mustards in or on foods without apparent adverse effects on man, and because, as a consequence, they have been generally recognized as safe, there have been few well-defined toxicological studies. The Select Committee is of the opinion that the mustards are examples of substances where more definitive toxicological studies on representative samples of the products actually used by the food industry would be useful in confirming the apparent absence of adverse health effects when they are used in or on foods. Moreover, in products like the mustards, which consist of variable mixtures of mustard seeds containing several chemical entities, the toxicological study of the products as actually used, at levels that approximate or exceed those now in current use, would be of greater significance and usefulness than the separate study of constituents known or assumed to be present.

The Select Committee has weighed the foregoing data and concludes that:

There is no evidence in the available information on allyl isothiocyanate, p-hydroxybenzyl isothiocyanate, and brown and yellow mustard 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 the future.
VI. REFERENCES CITED


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