EVALUATION OF THE HEALTH ASPECTS OF
AGAR-AGAR AS A FOOD INGREDIENT

DECEMBER, 1973

Prepared for

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

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Life Sciences Research Office
Federation of American Societies
for Experimental Biology
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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) 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 experience 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. JeJeff 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 agar-agar 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, including current literature citations obtained through Toxline* and Medline*, available as of December, 1973. Agar-agar 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 April 1, 1973).

As indicated in the Food, Drug, and Cosmetic Act [21 USC 321(s)], GRAS substances are exempt from the requirement of premarketing 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 conclusion 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 conclusions, 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 reconducted, using

*Nationwide online bibliographic retrieval systems initiated by the National Library of Medicine, Bethesda, Maryland.
new technologies that are continually being evolved. 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 agar-agar and submits its interpretation and assessment in this report, which is intended for the use of FDA in determining the future status of this substance under the Federal Food, Drug, and Cosmetic Act.

II. BACKGROUND INFORMATION

Agar-agar is a complex high molecular weight polysaccharide, primarily of galactose, extracted from several genera of red algae, notably Gelidium, Gracilaria, and Suhria. At least 14 other genera of red algae are used as commercial sources of agar-agar and related agar-like substances (1). The algae are harvested, dried, baled, and shipped to processing plants. The material is then washed and cooked in a series of extraction procedures. The cooking liquor is filtered, allowed to gel, and subsequently dried to yield agar flakes (2). The flakes are further dried to a moisture content of 20 percent. Agar-agar is marketed as flakes or powder in several grades of purity (1).

Agar-agar is composed of linear sulfated polysaccharide chains with alternating α and β linkages (3). Structural differences occur depending upon the genus from which the agar-agar is extracted, the time of year in which the algae are harvested, and the stage of growth of the plant. In general, three types of agar-agar exist: (a) neutral agarose, (b) pyruvated agarose with little sulfation, and (c) sulfated galactan with few or no 3,6-anhydro-L-galactose or 4,6-0-(1-carboxyethylidene)-D-galactose residues (3). The molecular weight of agar-agar varies with the grade, being 5,000 to 30,000 in the usual form but up to 160,000 in laboratory extracted types (1).

The Food Chemicals Codex specifies that food grade agar-agar should contain no more than 3 ppm of arsenic, 10 ppm of lead, and 40 ppm of heavy metals as lead (4). Agar-agar is insoluble in cold water or ethyl alcohol, but is slowly soluble in hot water and certain organic solvents. A 1.5 percent aqueous solution is clear, and forms a resilient gel when cooled to 32-39°C. Agar-agar is used extensively in the food industry as a gelling agent, and has been employed as a laxative. The discovery in 1658 of the usefulness of agar-agar as a gelling agent is attributed to a Japanese innkeeper; since that time it has been used in a number of
foods and has been available for food use in the United States since late in the nineteenth century (1).

Current use of agar-agar in foods, according to a recent survey by a National Research Council subcommittee (5), is given in Table I.

Table I

Agar-agar Content of Foods

<table>
<thead>
<tr>
<th>Food category</th>
<th>Usual use percent</th>
<th>Maximum percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baked goods</td>
<td>0.06</td>
<td>0.39</td>
</tr>
<tr>
<td>Frostings</td>
<td>0.16</td>
<td>0.27</td>
</tr>
<tr>
<td>Sweet sauces</td>
<td>0.20</td>
<td>0.25</td>
</tr>
<tr>
<td>Frozen dairy products</td>
<td>0.22</td>
<td>0.22</td>
</tr>
<tr>
<td>Processed fruits</td>
<td>0.15</td>
<td>0.15</td>
</tr>
<tr>
<td>Reconstituted vegetable protein</td>
<td>0.12</td>
<td>0.12</td>
</tr>
<tr>
<td>Soft candies</td>
<td>0.06</td>
<td>0.06</td>
</tr>
</tbody>
</table>

The total poundage used in foods in this country doubled between 1960 and 1970 (5). However, the Select Committee has no information to indicate whether the agar-agar content of the foregoing food categories has changed significantly in recent years.

III. CONSUMER EXPOSURE DATA

The National Research Council subcommittee (5) has supplied the following information on the possible daily intake of agar-agar by individuals in various age groups. The Select Committee has converted these figures to possible daily intakes per kilogram of body weight (Table II).
Table II
Possible Daily Intake of Agar-Agar

<table>
<thead>
<tr>
<th>Age group</th>
<th>Total</th>
<th>:Per kilogram of body weight*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average</td>
<td>Maximum</td>
</tr>
<tr>
<td></td>
<td>mg</td>
<td>mg</td>
</tr>
<tr>
<td>0-5 mos.</td>
<td>12.1</td>
<td>32.9</td>
</tr>
<tr>
<td>6-11 mos.</td>
<td>117.8</td>
<td>294.4</td>
</tr>
<tr>
<td>12-23 mos.</td>
<td>224.4</td>
<td>452.0</td>
</tr>
<tr>
<td>2-65+ yrs.</td>
<td>338.1</td>
<td>789.7</td>
</tr>
</tbody>
</table>

*Calculations based on an average weight of 60 kg for an adult (6) 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 (7).

The figures in Table II indicate that the groups consuming the largest amount per unit of body weight are children aged 6 to 23 months. It is also recognized that some older children as well could be consuming agar-agar at this higher level per kg of body weight since most individuals from age 2 to maturity will probably weigh less than 60 kg. Thus the daily intake of agar-agar for the children in this group could be significantly higher than the figures indicate.

However, such deviations from the figures in Table II must also be considered in respect to total quantity of agar-agar used in foods. The NRC subcommittee has pointed out that its calculations of intakes in most cases are overstated, often by considerable margins**. That they are overstated in the case of agar-agar is supported by the following calculations: The NRC subcommittee has provided data to show that the use of agar-agar in all forms for food purposes in the United States was 209,890 pounds (95,405 kg) in 1970 (5). This figure is reported to comprise between 60 and 70 percent of the total quantity used in food. On

**An explanation for such overstatements is detailed in Section XI, "Significance and Use of Data in Safety Evaluations," of the NRC subcommittee's report (5). 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 of the intake of substances used in food processing, food consumption data collected specifically for this purpose are needed."
the basis of 60 percent adjusted to 100 percent (159,008 kg) and a U.S. population of 210 million, the per capita per day average intake would be 2.2 mg. This suggests that not nearly enough agar-agar is used by the food industry to permit daily human intakes as high as indicated in Table II.

In view of these considerations, the Select Committee regards the figures given in Table II as levels that are unlikely to be achieved by any of the age groups.

The Joint FAO/WHO Expert Committee on Food Additives considers the acceptable daily intake of agar-agar for man as "not limited" (8).

IV. BIOLOGICAL STUDIES

The digestibility of agar-agar has been estimated in a number of studies. Nilson and Schaller (9) fed groups of six male, weanling rats diets containing 0 to 30 percent agar-agar for 10 weeks. Irrespective of levels fed, the digestibility was estimated to be 28 percent. In a similar study, Booth et al. (10) found the digestibility to be 21 percent in rats when agar-agar was fed at a level of 15 percent in the diet. By contrast, Ariyama and Takahasi (11) reported that agar-agar had no nutritive value for the rat. No direct information is available concerning the form in which agar-agar is absorbed or the nature of its digestive products. It is likely, however, that the complex polysaccharide is first digested to simpler sugars in the intestine prior to absorption.

While no acute oral toxicity studies have been reported, it should be noted that agar-agar has been used for many years as a gelling and bulking agent in semisynthetic diets for animal feeding studies. In most studies the agar-agar is present as 2 to 5 percent of the diet. No significant effects have been observed on growth, reproduction, or cytopathology (1). Because this material is often used in experimental diets, including controls, pathological disturbances are likely to have been noted if they occurred.

In studies in which agar-agar was fed to rats at dietary levels up to 30 percent, no significant pathological effects have been reported. In one experiment, male weanling rats were fed levels of agar-agar up to 30 percent of the diet for 10 weeks with no observable effect on growth (9). Ershoff and McWilliams (12) fed weanling female rats diets containing 10
percent agar-agar and noted only a slight depression in growth rate. However, Wierda (13,14) observed a thickening of the small intestine and an increase in length of the large intestine in rats fed high levels of agar-agar. In one study (13), 10 and 30 percent diets were fed to rats for 40 weeks; in a subsequent study (14), 50 weanling rats were fed a 30 percent agar-agar diet for 44 weeks. In both cases, the only change noted was an increase in intestinal weight and length. Increases in intestinal weight, due to mucosal rather than to muscular growth, also occurred at high levels of agar-agar after shorter feeding times; such changes were observed when a 25 percent agar-agar diet was fed to rats for four weeks (15). These changes are presumably related to the low digestibility and gelling properties of agar-agar. The increased bulk would cause these effects, and concentrations of 25 percent or more of agar-agar in the diet appear to be needed to produce them.

Schulz and Thomas (16) found that 25 percent of agar-agar in the diet of rats had no effect on the retention of lipids when compared to sucrose feeding. Thus it appears that the bulking effect of agar-agar does not prevent lipid absorption.

Other experimental animal studies yield analogous data. For example, rabbits showed increased growth rates, compared to controls, when fed 20 percent agar-agar diets for 40 days (17). At 2 percent of the diet, chicks showed no effect after 20 days (18). Cats grew at a normal rate and no gross pathological changes were observed when they were fed 5 percent agar-agar in the diet for one year beginning at 4 weeks of age (19).

No evidence of fetal toxicity was noted when 0.2 ml of a one percent agar-agar solution was administered intraperitoneally to mice each day from the 11th to the 15th day of gestation. Similar treatments during the 4th to 8th days of gestation increased resorptions by 15 percent (20). Oral administration of solutions containing one percent and 10 percent agar-agar from day 11 to day 15 of gestation produced no effect on the fetus and did not increase the rate of fetal resorption.

However, teratologic evaluations of agar-agar conducted recently by the Food and Drug Research Laboratories (21) have provided some evidence of maternal toxicity in two of the four species tested. Oral administration of up to 1140 mg per kg daily in rats (day 6 through day 15 of gestation), and up to 650 mg per kg daily in hamsters (day 6 through day 10 of gestation), had no clearly discernible effect on nidation or on maternal or fetal survival. The number of abnormalities seen in soft
and skeletal tissues of the test groups did not differ from the number occurring spontaneously in the sham-treated controls. Oral administration of up to 329 mg per kg daily in mice (day 6 through day 15 of gestation), and up to 118.5 mg per kg daily in rabbits (day 6 through day 18 of gestation), also elicited no discernible effect on nidation or on maternal or fetal survival, and resulted in no increase in the number of abnormalities in the soft or skeletal tissues of the offspring. However, when the dose was increased to 1570 mg per kg in mice and to 400 mg per kg in rabbits, there was a significant increase in mortality among the treated dams and a decrease in the pregnancy rate of surviving dams. In the case of mice, there was also a marked increase in the number of resorption sites in females examined at term, and the live fetuses showed significant retardation in maturation. There was no evidence of a teratogenic effect of agar-agar on mice or rabbits even at the highest dose level. The Food and Drug Research Laboratories' report indicated that the behavior of agar-agar in their teratologic tests was similar to that found previously with other high molecular weight polysaccharides. In their opinion, the administration of a dose of agar-agar amounting essentially "to an LD₅₀ dose even though distributed over a 10-day period," resulted "in a predictable effect on both the pregnant animal and on the embryos present in the uterus" (21).

Feeding studies designed to elicit evidence of mutagenicity have not been reported.

No specific studies of agar-agar are available in which the material was fed for more than one year to determine toxicity. However, it has been general practice to use agar-agar in animal diets as a gelling and bulking agent at levels ranging from 2 to 5 percent, and no adverse effects appear to have been reported in the numerous lifetime animal studies that have been conducted over the past several decades. Moreover, agar-agar has been used in basal diets fed to animals in numerous carcinogenicity studies, and the Select Committee is not aware of any reports of increased tumor incidence. Based upon the normal food intake of the rat, a concentration of 5 percent agar-agar in the diet is equivalent to 3 g per kg per day, a level considerably greater than that consumed by man on a body weight basis.

Since agar-agar serves as the gelling agent for media used in bacteriological investigations, the effect of this material on the growth of microorganisms has been intensively studied. For example, De and
Guha (22) reported that an 82 percent agar-agar diet provided a favorable medium for the growth of intestinal microflora. On the other hand, variable results were found in studies performed in vitro in which the effect of agar-agar on the inhibition of microbial growth by various antibiotics was reported (23, 24). However, at levels generally used in foods, there is no evidence that agar-agar affects microbial metabolism in the alimentary tract.

Since 1905, agar-agar has been used by man as a laxative, in doses ranging from 4 to 15 g per day with no apparent adverse effect (25). These doses are equivalent to 67 to 250 mg per kg per day, levels that are considerably above those estimated as the daily intake from food (Table II).

V. OPINION

Agar-agar has relatively little effect when added to the diets of animals in amounts considerably greater than those present in the human diet. The observed increases in intestinal weight and length in animals appear to be related to the bulking and hydrocolloidal properties of the material, and these changes occur only at relatively high concentrations of agar-agar.

Although no specific studies of the carcinogenicity or other long-term investigations of agar-agar have been made, this material has a long history of use as a gelling agent and bulk component of experimental animal diets. Because 2 to 5 percent of this material has been used routinely in control diets in numerous studies without reported significant effects, it is reasonable to conclude that even at these high levels, agar-agar produces no significant chronic effects.

However, there is one report that agar-agar, fed at relatively high levels (400 to 1570 mg per kg per day), is lethal to many pregnant mice and rabbits but not to pregnant rats and hamsters fed at equivalent levels (650 to 1140 mg per kg per day). Significant numbers of maternal deaths occurred in pregnant mice and rabbits fed agar-agar at levels 118 fold greater (mice) and 30 fold greater (rabbits), than the maximum level estimated to be consumed by adults (13.2 mg per kg per day) in the daily diet, but no toxic effects were observed in pregnant mice and rabbits fed levels 25 fold greater (mice) and 9 fold greater (rabbits)
than the estimated adult human intake level. With respect to these comparisons it should be emphasized that the Select Committee believes the intake estimate of 13.2 mg per kg per day (Table II) is overstated by a considerable margin, which could make the foregoing differences in each case even larger.

It is noteworthy that similar toxic effects have been observed in identical tests on a number of other polysaccharides (gum arabic, sterculia gum, carob bean gum, guar gum, gum ghatti, gum tragacanth, carrageenan, propylene glycol alginate, and methyl cellulose) fed at very high levels. The relative sensitivity of the several animal species to these effects varies depending on the particular polysaccharide tested, but in all cases very large doses are required. Until these effects have been adequately explained, it appears to be inappropriate to conclude that unrestricted use of such substances in food would be without hazard.

Agar-agar is a product extracted from marine algae. The possibility exists that harmful concentrations of certain metals such as mercury, may be accumulated in the commercial product if algae are harvested from coastal waters contaminated with significant levels of such heavy metals (26). Current specifications for food grade agar-agar (4) place a limitation on the content of "heavy metals as lead." Because modern methods of analysis are capable of distinguishing between and measuring the amounts of the several metal elements, it would appear advisable to make the specifications for agar-agar more specific with respect to allowable concentrations of potentially toxic heavy metals, such as mercury.

The Select Committee has weighed the foregoing and concludes that:

There is no evidence in the available information on agar-agar that demonstrates, or suggests reasonable grounds to suspect, a hazard to the public when it is used at levels that are now current and in the manner now practiced. However, it is not possible to determine without additional data, whether a significant increase in consumption would constitute a dietary hazard.
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Date

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