EVALUATION OF THE HEALTH ASPECTS OF RIBOFLAVIN AND RIBOFLAVIN-5'-PHOSPHATE AS FOOD INGREDIENTS

1979

Prepared for

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

Contract No. FDA 223-75-2004
EVALUATION OF THE HEALTH ASPECTS OF RIBOFLAVIN AND
RIBOFLAVIN-5'-PHOSPHATE AS FOOD INGREDIENTS

1979

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

Contract No. FDA 223-75-2004

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 concerning the health aspects of using the Generally Recognized as Safe (GRAS) or prior sanctioned food substances as food ingredients, being made by the Federation of American Societies for Experimental Biology (FASEB) under contract no. 223-75-2004 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.

Kenneth D. Fisher, Ph.D., Director
Life Sciences Research Office
FASEB
CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Introduction</td>
<td>1</td>
</tr>
<tr>
<td>II. Background information</td>
<td>3</td>
</tr>
<tr>
<td>III. Consumer exposure data</td>
<td>5</td>
</tr>
<tr>
<td>IV. Biological studies</td>
<td>10</td>
</tr>
<tr>
<td>V. Opinion</td>
<td>17</td>
</tr>
<tr>
<td>VI. References cited</td>
<td>18</td>
</tr>
<tr>
<td>VII. Scientists contributing to this report</td>
<td>24</td>
</tr>
</tbody>
</table>
I. INTRODUCTION

This report concerns the health aspects of using riboflavin and riboflavin-5'-phosphate as food ingredients. It 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 1974.* In addition, the Select Committee was provided with a review prepared by LSRO, of the more recent pertinent reports on these compounds (2).* 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 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; recent literature searches by the Toxicology Information Response Center, Oak Ridge, Tennessee; 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, an announcement was made in the Federal Register of January 12, 1979 (44 FR 2687-2690) 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 riboflavin and riboflavin-5'-phosphate as food ingredients. The Select Committee received no requests for such a hearing.

As indicated in the Food, Drug, and Cosmetic Act [21 USC 321 (s)], GRAS substances are exempt from the premarketing clearance that is required for food additives. It is stated in the Act and in the Code of Federal Regulations (3) [21 CFR 170.3 and 170.30] 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. These sections of the Code also indicate 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 (3) recognizes further [21 CFR 170.30] that it is impossible to provide assurance that any substance is absolutely safe for human consumption.

*These documents (PB 241-964/6 and PB 275-753) are available from the National Technical Information Service, U.S., Department of Commerce, P.O. Box 1553, Springfield, Virginia 22161.
The Select Committee on GRAS Substances of LSRO is making its evaluation of these substances in full recognition of the foregoing provisions. In reaching its conclusions on safety, the Committee, in accordance 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. While the Committee realizes that a conclusion based on such reasoned judgment is expected even in instances where the available information is qualitatively or quantitatively limited, it recognizes that there can be instances where, in the judgment of the Committee, there are insufficient data upon which to base a conclusion. The Committee is aware that its 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 riboflavin and riboflavin-5'-phosphate 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

Riboflavin, 6,7-dimethyl-9-(1'-d-ribityl) isoalloxazine, or a conjugated form, is found in minute amounts in practically all tissues and cells. Milk, egg white, liver, heart, kidney, leafy vegetables and especially yeast are good sources of the vitamin. Free riboflavin has been demonstrated in the retina, whey and urine (4). The forms normally found in nature are riboflavin-5'-phosphate [flavin mononucleotide (FMN)] and flavin-adenine-dinucleotide (FAD), both of which serve as coenzymes for a number of oxidases and dehydrogenases, collectively known as flavoprotein enzymes (5). Riboflavin deficiency produces a variety of metabolic impairments manifested by subnormal growth, corneal vascularization, dermatitis, alopecia, fatty liver, scrotal dermatitis, and various oral and facial lesions (6,7).

Riboflavin is a yellow to orange-yellow crystalline powder having a slight odor. It decomposes at about 280°C and is neutral in a saturated solution. It is only slightly soluble in water at room temperature, less soluble in alcohol and insoluble in ether and chloroform. It is, however, very soluble in dilute alkali. Light has no effect on the dry form but causes breakdown of riboflavin in solution. Riboflavin-5'-phosphate (sodium salt) is hygroscopic and much more water soluble (approximately 3 percent) than free riboflavin. It, too, is affected by light when in solution, but not in its dry form (8).

The Food Chemicals Codex (8) specifies that preparations of riboflavin used in foods must contain from 98.0 to 102.0 percent of the vitamin calculated on the dry basis. It must lose not more than 1.5 percent by weight on drying at 105°C for 2 hours and must have a residue of less than 0.3 percent after ignition. Its specific rotation must be between -112° and -122°. Specifications for sodium riboflavin-5'-phosphate require that it be equivalent to 70 to 75 percent riboflavin with a specific rotation between +37.0° and +42.0°. The following limits must not be exceeded: free phosphate, 1 percent; free riboflavin, 6 percent; loss on drying, 7 percent; riboflavin diphosphate, 6 percent (calculated as riboflavin); residue on ignition, 25 percent. The pH of 1:100 solution must be 5.0 to 6.5.

Riboflavin [21 CFR 182.5695] and riboflavin-5'-phosphate [21 CFR 182.5697] are classified by FDA as nutrients and/or dietary supplements with GRAS status (3). When used to enrich various foods such as bakery, cereal and pasta products, limits are specified and vary from 1.1 mg per pound (2.4 mg per kg) for bread, rolls and buns [21 CFR 136.115] to 1.7 to 2.2 mg per pound (3.7 to 4.8 mg per kg) for macaroni and noodle products [21 CFR 139.115; 139.117; 139.122; 139.155]. Riboflavin is also listed as a color additive [21 CFR 73.450] which is exempt from certification.
Riboflavin is incorporated in tablets, capsules and liquids, singly or in combination with other dietary supplements, for over-the-counter sale. The Physicians' Desk Reference (9) lists over 100 pharmaceutical and vitamin preparations containing riboflavin sold in the United States.
III. CONSUMER EXPOSURE DATA

This report is concerned only with riboflavin and riboflavin-5'-phosphate as GRAS substances added to foods. In keeping with the fact that they are vitamins, these substances are normally added in small amounts. Table I is based on information supplied to a subcommittee of the National Research Council (NRC) (10) by manufacturers in 1970 who reported adding these substances to at least one food in a given category. Riboflavin, rather than its phosphate, was the vitamin form utilized almost exclusively. The latter form was added only to milk products at a level of 1 ppm.

The level of addition shown in Table I does not indicate that any particular product contains that amount of riboflavin nor does inclusion of a food category within this table mean that the compound is present in a majority of the foods in that category. Actually, many of the foods within the listed food categories may contain much lower levels or none of the added vitamin. The data in this table probably represent overestimates of the actual average level of added riboflavin.

Nevertheless, the NRC subcommittee (10) has utilized these values to calculate the possible average daily intake of vitamins in several age groups. For this purpose, the subcommittee has used the values shown in Table I, together with data on the mean frequency with which foods in each category are consumed (provided by the Market Research Corporation of America) and the mean portion size of these various foods (as determined by the U.S. Department of Agriculture). The resulting calculations yield the following possible average daily intakes of added vitamin (total of riboflavin and riboflavin-5'-phosphate) for the different age groups: 0 to 5 months, 0.8 mg; 6 to 11 months, 2.2 mg; 12 to 23 months, 2.9 mg; and over 2 years of age, 4.5 mg.

The NRC subcommittee (10) has recognized the shortcomings of these calculations and has cautioned that their application will result in a considerable overstatement of average intakes for most GRAS substances.

An alternative approach for estimating the intake of added GRAS substances is based on the total amount added annually to food by the manufacturers. The survey of the NRC subcommittee (10) revealed that 55,200 kg of riboflavin and 18 kg of riboflavin-5'-phosphate had been added to foods in 1970 by industrial respondents to the survey. The NRC estimated that the reported amounts represented only 60 percent of the actual industrial use; corrected to 100 percent or 91,000 kg*, the average per capita daily intake of riboflavin added to food would be 1.2 mg. This

---

*A resurvey in 1975 reported annual use of 70,000 kg of riboflavin (58).
TABLE I

Level of Addition of Riboflavin to Foods by Food Category

<table>
<thead>
<tr>
<th>Food category</th>
<th>Riboflavin mg per kg (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baked goods, baking mixes</td>
<td>2</td>
</tr>
<tr>
<td>Breakfast cereals</td>
<td>21</td>
</tr>
<tr>
<td>Grain products such as pastas or rice dishes</td>
<td>3</td>
</tr>
<tr>
<td>Milk products</td>
<td>5</td>
</tr>
<tr>
<td>Processed fruits, juices and drinks</td>
<td>3</td>
</tr>
<tr>
<td>Meat products</td>
<td>7</td>
</tr>
<tr>
<td>Poultry products</td>
<td>30</td>
</tr>
<tr>
<td>Fish products</td>
<td>30</td>
</tr>
<tr>
<td>Condiments, relishes, salt substitutes</td>
<td>2</td>
</tr>
<tr>
<td>Sweet sauces, toppings, syrups</td>
<td>5</td>
</tr>
<tr>
<td>Snack foods</td>
<td>2</td>
</tr>
<tr>
<td>Beverages, nonalcoholic</td>
<td>17</td>
</tr>
<tr>
<td>Reconstituted vegetable proteins</td>
<td>2</td>
</tr>
<tr>
<td>Dairy products analogs</td>
<td>7</td>
</tr>
<tr>
<td>Baby food baked goods</td>
<td>29</td>
</tr>
<tr>
<td>Baby food cereals</td>
<td>9</td>
</tr>
<tr>
<td>Baby food formulas</td>
<td>1</td>
</tr>
<tr>
<td>Baby food combination dinners</td>
<td>7</td>
</tr>
</tbody>
</table>

Level of addition of riboflavin and riboflavin-5'-phosphate is the weighted mean of the levels reported by manufacturers as their usual addition to one or more products in a food category. For discussion of weighted mean see Section X and Exhibit 50 of reference 10.
amount is believed to represent a more reasonable estimate of the average daily intake than that calculated as described above. The amount contributed by riboflavin-5'-phosphate was less than 0.001 mg daily.

A comparison of 1970 and 1960 usage of riboflavin for respondents reporting data for both years indicates a 1.5-fold increase in usage during the 10 year period (10).

The U.S. production of riboflavin, its esters and salts in 1973 was 335,000 kg (11) and imports in 1975 were 113,000 kg (12). These are the latest years for which such data are available. Unfortunately, differences in reporting procedures make it impossible to determine the total production and import values for the same year. Virtually all imports and domestic production was presumably used in foods or in various vitamin formulations. Assuming total consumption is about 448,000 kg, the daily per capita intake would be approximately 6 mg. This suggests that considerably more riboflavin is consumed in vitamin preparations than in foods to which riboflavin is added as a GRAS ingredient.

Although riboflavin is relatively stable to heat, losses up to 20 percent may occur during food preparation from exposure to light and extraction of the vitamin in the cooking water (13). Milk in glass containers exposed to bright sunlight may lose as much as 85 percent of its riboflavin in 2 hours (14).

The Recommended Dietary Allowance (RDA) for riboflavin is based upon recommendations of the Food and Nutrition Board (FNB) of the National Academy of Sciences/National Research Council. The FNB has attempted to relate the riboflavin allowances to parameters other than age or weight of the individual. In successive editions of its "Recommended Dietary Allowances," it utilized protein allowance (1958) (15), energy intake (1964) (16) and metabolic body size (1968) (17) as bases for determining riboflavin requirements. In the latest edition (1974) (18), the FNB concludes that the allowances calculated by these three methods do not differ significantly and reverts to its earlier method of relating the riboflavin requirement to energy intake. It recommends 0.6 mg per 1000 kcal for persons of all ages engaged in normal activity with a daily supplement of 0.3 mg for pregnant and 0.5 mg for lactating women. These recommendations are generally slightly lower than those submitted by other nutritional advisory bodies (Table II) (19).

In an attempt to assess the nutritional status of the U.S. population, the National Center for Health Statistics (20) in 1971 to 1974 estimated the dietary intake of certain nutrients (including riboflavin) according to age, sex, race and income. Over 20,000 persons from 1 to 74 years of age were surveyed. The mean intake of riboflavin was 1.92 mg per day with a median level of 1.69 mg. Based on energy intake, the mean level was 0.96 mg per 1000 kcal or 160 percent of the recommended allowance. The
## TABLE II

Recommended Allowances for Riboflavin (mg per day)\textsuperscript{a}

<table>
<thead>
<tr>
<th>Subjects</th>
<th>RDA</th>
<th>WHO/FAO\textsuperscript{b}</th>
<th>Committee for Revision of Dietary Standards, Canada\textsuperscript{b}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infants (0-1 yr)</td>
<td>0.4-0.6</td>
<td>0.5</td>
<td>0.4-0.6</td>
</tr>
<tr>
<td>Children</td>
<td>0.8-1.5</td>
<td>0.8-1.6</td>
<td>0.8-1.5</td>
</tr>
<tr>
<td>Teenagers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>1.5-1.8</td>
<td>1.7-1.8</td>
<td>1.7-2.0</td>
</tr>
<tr>
<td>Female</td>
<td>1.3-1.4</td>
<td>1.4-1.5</td>
<td>1.3-1.4</td>
</tr>
<tr>
<td>Adults</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>1.5-1.8</td>
<td>1.8</td>
<td>1.7-2.0</td>
</tr>
<tr>
<td>Female</td>
<td>1.1-1.4</td>
<td>1.3</td>
<td>1.2-1.3</td>
</tr>
<tr>
<td>Pregnant women</td>
<td>1.5-1.7</td>
<td>1.5-1.7\textsuperscript{c}</td>
<td>1.5-1.7</td>
</tr>
<tr>
<td>Lactating women</td>
<td>1.7-1.9</td>
<td>1.7-1.9</td>
<td>1.8-2.0</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Adapted from reference 19

\textsuperscript{b} Recommended intake

\textsuperscript{c} Latter half of pregnancy
RDA of 0.6 mg per 1000 kcal was exceeded by every age, sex, race and income group. The lowest intake of any of these groups was 0.71 mg per 1000 kcal for black males 18 to 19 and 35 to 44 years of age, with incomes below the poverty level. Data on pregnant or lactating women were not reported.
IV. BIOLOGICAL STUDIES

Absorption, distribution and metabolism

Riboflavin is rapidly absorbed from the intestinal tract. In man, absorption is limited mainly to the upper portion of the small intestine (21), while in the rat the site of maximum absorption appears to be the lower ileum (22,23). Orally administered riboflavin-5'-phosphate (FMN) is converted to riboflavin in the intestine (24,25). It appears in the plasma (26), urine (26,27), and feces (27) largely, if not entirely, as the free vitamin. According to Jusko and Levy (28), FMN is dephosphorylated in the intestinal lumen, rephosphorylated in the intestinal mucosa, transported to the liver where it is again rapidly dephosphorylated, circulated in the blood as free riboflavin, and finally excreted in this form in the urine.

Morrison and Campbell (29) gave human subjects 1 to 20 mg riboflavin (about 0.02 to 0.35 mg per kg) by mouth in single or divided doses. The urinary excretion remained a constant percentage of the dose, suggesting absorption by passive diffusion. With larger doses, however, there was a decrease in the relative amounts excreted in the urine (26,30), and ingestion of more than 50 mg of riboflavin or FMN caused no further increase in the urinary excretion (26). Such a limited capacity to absorb riboflavin implies a special, saturable, absorptive mechanism (26,28). This capacity was estimated by Stripp (26) to be approximately 14 to 18 mg of riboflavin or FMN and by Mayersohn et al. (30) as 11 to 12 mg.

Urinary excretion of riboflavin after oral administration of large doses of FMN frequently shows two maxima (28). It was originally believed that the second maximum resulted from enterohepatic cycling in man. However, subsequent studies revealed that the biliary excretion of riboflavin in man was negligible and the secondary maximum was probably due to an effect of bile in enhancing absorption of riboflavin (31). Bile salts administered with doses of 5 to 30 mg (about 0.08 to 0.5 mg per kg) of riboflavin or 30 mg FMN in adult men increased the total amount of riboflavin excreted in the urine (30).

Riboflavin and FMN are rapidly metabolized but little is known of their fate. When 10 to 15 mg per kg body weight were administered to rats orally or intraperitoneally, only about one-third could be recovered 24 hours later in the urine and feces and none from the animal's carcass (25). Christensen (32) believed this rapid disappearance of riboflavin from the body reflected an active metabolism, probably centered in the liver. To demonstrate the major role of the liver, he excluded the portal circulation in rats by ligating the common hepatic artery and the portal vein. The portal blood was pumped back into the
right femoral vein. Two mg FMN per kg body weight were then injected into the carotid artery. Fifteen minutes after injection, blood riboflavin of the test animals was 7 to 9 times, and 30 minutes later, 30 to 40 times that of the control animals. The test animals lived for about 1 hour after injection.

Only a few degradative products of riboflavin and FMN have been detected although others are suspected. Yang and McCormick (33) identified lumichrome and lumiflavin in the feces of young adult rats after intraperitoneal injection of $^{14}$C-labeled-riboflavin. West and Owen (34) recovered hydroxyethylflavin, formylmethylflavin and an unidentified compound from the urine of male subjects given 1 g (about 15 mg per kg) of riboflavin by mouth. Both laboratories (33,34) attributed the riboflavin metabolites to bacterial action in the gastrointestinal tract. Christensen (35) also detected lumichrome as well as four other unidentified products in the urine and feces of rats given labeled riboflavin orally or subcutaneously. He believed, however, that these were produced by the rat tissues and not by bacterial action, for no significant decomposition of the riboflavin molecule had been demonstrated during incubation with rat intestinal contents (25).

**Acute toxicity**

Table III summarizes the available data on the acute toxicity of riboflavin and FMN. The most extensive study was that of Unna and Greslin (36) who administered riboflavin and sodium riboflavinate (presumably sodium salt in FMN) orally, subcutaneously and intraperitoneally to a total of 400 adult rats. Oral administration of 10 g per kg body weight of riboflavin or sodium riboflavinate was tolerated without evidence of toxic effects, as was the subcutaneous injection of 5 g per kg riboflavin. However, it is to be noted that both compounds were administered as suspensions and the amount absorbed is not known. Riboflavin is only slightly water soluble (0.1 g per l). The intense yellow color of the rats' feces and the massive deposits of riboflavin at the sites of injection indicated incomplete absorption by both the oral and subcutaneous routes. Absorption after intraperitoneal injection seemed relatively complete, for only traces of riboflavin could be detected in the peritoneal cavity 3 days after injection and no difference in toxicity from sodium riboflavinate was apparent. The slight solubility of riboflavin precluded determination of its intravenous toxicity. Sodium riboflavinate is more water soluble (20 g per l) than riboflavin and is more readily absorbed after subcutaneous injection.

Lipkan (37) also determined the acute toxicity of riboflavin injected subcutaneously and intraperitoneally into rats. The LD$_{50}$ after subcutaneous injection (8 g per kg) compared reasonably well with that reported by Unna and Greslin (36)
### TABLE III

Acute Toxicity of Riboflavin Congeners

<table>
<thead>
<tr>
<th>Substance</th>
<th>Animal</th>
<th>No.</th>
<th>Route</th>
<th>Dosage mg/kg body wt.</th>
<th>Measurement</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Riboflavin</td>
<td>Rat</td>
<td>a</td>
<td>Oral</td>
<td>&gt;10,000</td>
<td>LD₅₀</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td></td>
<td>a</td>
<td>S.C.</td>
<td>&gt; 5,000</td>
<td>LD₅₀</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>S.C.</td>
<td>5,000</td>
<td>LD₀</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>S.C.</td>
<td>8,000</td>
<td>LD₅₀</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td></td>
<td>a</td>
<td>I.P.</td>
<td>560</td>
<td>LD₅₀</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>I.P.</td>
<td>3,000</td>
<td>LD₀</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>I.P.</td>
<td>4,000</td>
<td>LD₅₀</td>
<td>37</td>
</tr>
<tr>
<td>Mouse</td>
<td>10</td>
<td></td>
<td>S.C.</td>
<td>30</td>
<td>LD₀</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td></td>
<td>S.C.</td>
<td>60</td>
<td>LD₁₀₀</td>
<td>38</td>
</tr>
<tr>
<td>Dog</td>
<td>3</td>
<td></td>
<td>Oral</td>
<td>&gt; 2,000</td>
<td>LD₀</td>
<td>36</td>
</tr>
<tr>
<td>Sodium Riboflavinate</td>
<td>Rat</td>
<td>a</td>
<td>Oral</td>
<td>&gt;10,000</td>
<td>LD₅₀</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td></td>
<td>a</td>
<td>S.C.</td>
<td>790</td>
<td>LD₅₀</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td></td>
<td>a</td>
<td>I.P.</td>
<td>560</td>
<td>LD₅₀</td>
<td>36</td>
</tr>
<tr>
<td>FMN⁺</td>
<td>Mouse</td>
<td>6</td>
<td>Oral</td>
<td>&gt; 6,000</td>
<td>LD₅₀</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td></td>
<td>24</td>
<td>S.C.</td>
<td>800</td>
<td>LD₅₀</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td></td>
<td>24</td>
<td>I.V.</td>
<td>600</td>
<td>LD₅₀</td>
<td>39</td>
</tr>
</tbody>
</table>

*Number in each group not stated. Total of 400 rats for the 6 groups.

⁺Mono-diethanolamine salt.
 (>5 g per kg), but the results of intraperitoneal injection differed markedly between the two laboratories. The LD<sub>50</sub> determined by Lipkan (37) (on five rats) was 4 g per kg and that by Unna and Greslin (36), 560 mg per kg body weight. Experimental details are lacking in Lipkan's report.

The injection of toxic doses of either riboflavin or its sodium salt produced anuria for 1 or 2 days, with subsequent scanty excretion of deep yellow-colored urine (36). The rats became listless, lost weight rapidly and died within 5 days. Numerous bright yellow crystals, presumably riboflavin, were found in the kidneys. Concretions were also found in the kidneys of all rats killed either by intraperitoneal riboflavin injection or by sodium riboflavinate given subcutaneously or intraperitoneally. No crystals were detected in the kidneys of rats receiving 10 g riboflavin per kg by mouth or 5 g per kg subcutaneously.

Mice receiving 60 or 120 mg per kg riboflavin subcutaneously showed increased neuromuscular sensitivity and died in about 30 minutes (38). Mild hypothermia was the only ill effect noted at a dosage of 30 mg per kg. Three dogs received 2 g of riboflavin per kg by mouth without evidencing any toxic signs (36). Upon sacrifice after 8 hours, 36 hours and 17 days, respectively, their kidneys showed no pathological change.

Short-term studies

Demole (40) in 1938 reported no toxic effects in various species (mouse, rat, dog, cat, rabbit, frog, goldfish, axolotl) receiving from 1 to 400 mg riboflavin per kg daily for 3 to 10 days. The riboflavin was administered orally or by subcutaneous, intravenous, intramuscular, or intraperitoneal injection. Each test group was small, consisting of only one to five members.

Four dogs (breed not specified), 10 weeks of age, were fed 25 mg riboflavin per kg body weight daily for 5 months with two litter-mates serving as controls (36). Their growth was normal and no toxic signs were observed. Macroscopic examination of the organs after the animals' sacrifice failed to reveal any abnormalities.

The highly soluble mono-diethanolamine salt of FMN was fed to groups of 10 weanling Sprague-Dawley female rats 5 days weekly for 29 weeks at dose levels of approximately 5, 20, 50, and 200 mg per kg body weight (39). No deleterious effects on growth or blood cells were noted at the 5 or 20 mg per kg levels. The 50 mg per kg dose did not impair growth but caused a slight decrease in hemoglobin concentration. At doses of 200 mg per kg, two of ten rats died and the surviving animals showed slight anemia and decreased growth. Rabbits received 5 or 50 mg per kg body weight of the compound by intravenous or intra-
muscular injection, 5 days weekly for 3 weeks (41). No toxic effects were noted after intramuscular injection. One of four rabbits died with evidence of kidney damage after seven intravenous injections of the higher dose level.

In man, large doses of riboflavin have been used in the treatment of various clinical conditions. Shepard et al. (42) gave 4 g per day (about 200 mg per kg) for 9 days to a 7-year-old patient with primary hyperoxaluria. The treatment had no effect on the urinary excretion of oxalate but produced no toxic effects. Welsh and Ede (43) studied 310 patients with psoriasis to whom they administered oral daily doses of 10 to 60 mg (about 0.15 to 1 mg per kg) FMN or 20 to 1000 mg (about 0.3 to 15 mg per kg) of riboflavin for periods up to 42 months. They concluded that the treatment was without significant benefit. Although the highest dose level represented approximately 500 times the Recommended Dietary Allowance, no toxicity was reported.

Sensitivity to riboflavin appears to be rare. The Select Committee could find only two possible cases reported in the literature. A 22-year-old female patient with a history of frequently recurring eczema, received an injection (route not stated) of 10 mg riboflavin (about 0.15 mg per kg). Thirty minutes later, she experienced a marked reaction characterized by chills, edema, itching, eosinophilia and intense hyperemia (44). In a second case (45) a woman, 50 years of age, developed a severe pruritic dermatitis from "B-complex capsules." Discontinuance of the capsules resulted in the disappearance of all symptoms within 48 hours. A less severe eruption recurred whenever she consumed bakery products from flour enriched with thiamin, riboflavin and niacin. Which, if any, of these was the responsible agent was not investigated.

Long-term studies

The Select Committee is not aware of any studies in which riboflavin or FMN has been administered in large amounts over essentially the full lifespan of experimental animals. The closest approach was that of Unna and Greslin (36), who fed three generations of rats 10 mg riboflavin (about 50 mg per kg body weight) daily for 104 days, from the time of weaning to that of mating. No difference in the growth, development or reproductive performance was observed between the three generations of rats fed riboflavin and the control groups. Necropsies at the end of the feeding periods revealed no gross changes in the organs.

Reproduction

No difference was noted in the offspring of pregnant and lactating rats receiving diets containing 4 mg or 100 mg riboflavin per kg diet (daily intake of 0.4 and 10 mg per kg body weight, respectively) (46). The 25-fold increase in riboflavin
during the reproductive period had no effect on the litter size, growth until weaning, or vitamin requirements after weaning.

Leclerc (47) found no significant difference in the number per litter, mortality or weight gain of young Wistar rats whose mothers received 4 or 40 mg riboflavin per kg diet (0.4 and 4 mg per kg body weight daily) during pregnancy and lactation; nor was there any difference between the two groups in the riboflavin content of liver or of total carcass of the weanling rats. A daily intake of 0.4 mg per kg body weight appears adequate to saturate the tissues.

The ability of the human placenta to transfer riboflavin from maternal to fetal blood was investigated in 10 normal deliveries (48). Fetal blood contained about four times as much free riboflavin as maternal blood but only about half as much flavin adenine dinucleotide (FAD). Only small amounts of FMN were detected in both maternal and fetal bloods. The placenta rapidly split FAD to FMN and free riboflavin in vitro. The data suggest that the placenta removes FAD from maternal blood and converts it enzymatically to free riboflavin, which is then secreted into the fetal blood.

Carcinogenesis

Riboflavin deficiency has been reported to retard the growth of certain experimentally induced cancers. Morris and Robertson (49) found the growth and spread of mammary cancer to be markedly lower in riboflavin-deficient C3H mice than in controls. When the deficient diet was supplemented with riboflavin, the growth of the neoplasm was accelerated (50). More recently, it has been shown that riboflavin deficiency significantly prolonged the survival of Holzman rats in which Novikoff hepatoma had been grown intraperitoneally (51).

On the other hand, riboflavin deficiency enhances the carcinogenicity of certain azo dyes such as 4-dimethylaminoazobenzene (52). This has been attributed to the requirements of the azo dye reductase for flavin cofactors (53). Rat liver and cecal contents are rich sources of the enzyme. When low-riboflavin diets are fed, this enzymatic activity is markedly reduced at both sites, allowing more of the dye to retain its carcinogenic potential.

Teratogenesis

It is well known that maternal deficiency of riboflavin results in severe congenital malformations of the offspring (54). The Select Committee is not aware of studies relating large riboflavin intakes with teratogenesis.
Mutagenesis

Riboflavin (55) and FMN (56) were tested for mutagenic activity in a series of in vitro microbial assays with and without metabolic activation by mammalian systems. Both the plate and the suspension test procedures were utilized. The indicator organisms were Saccharomyces cerevisiae, strain D4 and Salmonella typhimurium, strains TA-98, -100, -1535, -1537, and -1538. Activating preparations were made from tissues of ICR random-bred adult male mice, Sprague-Dawley rats and Macaca mulatta monkeys. Neither compound exhibited mutagenic activity in any of the assays employed.

Special studies

Newberne et al. (57) found that exposure to riboflavin and light enhanced the toxicity of aflatoxin in the rat. Weanling male Sprague-Dawley rats were dosed intragastrically with 28 mg per kg of riboflavin and 30 minutes later with LD50 amounts of aflatoxin B1 or monocrotaline (7 and 70 mg per kg, respectively). Half of the rats were then exposed for 2 hours to radiation approximating natural light. After irradiation, animals were fed their respective diets for 14 days at which time they were sacrificed. The cumulative mortality after 2 weeks was 45 percent for rats receiving aflatoxin alone; 50 percent for aflatoxin and riboflavin; 57 percent for aflatoxin plus light; and 75 percent for aflatoxin, light and riboflavin. The addition of riboflavin had no significant effect on the mortality of rats dosed with monocrotaline.
V. OPINION

Riboflavin, an essential nutrient, is a constituent of two coenzymes: riboflavin-5'-phosphate [flavin mononucleotide (FMN)] and flavin adenine dinucleotide (FAD), which are essential components of a number of oxidative enzyme systems. Various foods such as bakery, cereal and pasta products are commonly enriched by the addition of 2 to 5 mg per kg product. Also, many commonly used vitamin supplements contain riboflavin. The amount of riboflavin-5'-phosphate added to food is miniscule.

The Recommended Dietary Allowance of riboflavin is 0.6 mg per 1000 kcal for persons of all ages with an additional 0.3 mg daily for pregnant and 0.5 mg for lactating women. A recent U.S. survey of over 20,000 persons, 1 to 74 years of age, revealed a mean average intake of 1.92 and a median of 1.69 mg per day.

The acute toxicity in animals of riboflavin or FMN given orally is extremely low, with LD₅₀ values several orders of magnitude greater than the dietary requirement or the estimated addition to food. The relative insolubility of riboflavin limits the absorption when large amounts are ingested. No reports have come to the attention of the Select Committee suggesting carcino- genic, mutagenic or teratogenic effects of riboflavin. Normal reproductive performance was observed in three generations of rats fed several hundred times their daily requirement. Toxic effects in man have not been reported apart from rare instances of sensitivity.

In the light of these considerations, the Select Committee concludes that:

There is no evidence in the available information on riboflavin or riboflavin-5'-phosphate 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


VII. SCIENTISTS CONTRIBUTING TO THIS REPORT

1. Members of the Select Committee on GRAS Substances:

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

Harry G. Day, Sc.D., Professor Emeritus of Chemistry, Indiana University, Bloomington, Ind.

Samuel J. Fomon, M.D., Professor of Pediatrics, College of Medicine, University of Iowa, Iowa City, Iowa.

Bert N. La Du, Jr., M.D., Ph.D., Professor and Chairman, Department of Pharmacology, University of Michigan Medical School, Ann Arbor, Mich.

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.

Michael B. Shimkin, M.D., Professor of Community Medicine and Oncology, School of Medicine, University of California, San Diego, La Jolla, Calif.

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), Life Sciences Research Office, Federation of American Societies for Experimental Biology, Bethesda, Md.

*Did not participate in final opinion reached in this report.
2. LSRO staff:

Kenneth D. Fisher, Ph.D., Director
Frederic R. Senti, Ph.D., Associate Director
Richard G. Allison, Ph.D., Staff Scientist
Sue Ann Anderson, Ph.D., Staff Scientist
Herman I. Chinn, Ph.D., Senior Staff Scientist
Andrew F. Freeman, Senior Staff Scientist
John M. Talbot, M.D., Senior Medical Consultant
Michael J. Wade, Ph.D., Staff Scientist

The Select Committee expresses its appreciation to Hoffmann-LaRoche, Inc., Nutley, N.J. 17110, who contributed information and data.

Report submitted by:

September 17, 1979

Date

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