EVALUATION OF THE HEALTH ASPECTS OF VITAMIN D₂
AND VITAMIN D₃ AS FOOD INGREDIENTS

1978

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 VITAMIN D₃ AND VITAMIN D₉ AS FOOD INGREDIENTS

1978

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>7</td>
</tr>
<tr>
<td>V. Opinion</td>
<td>22</td>
</tr>
<tr>
<td>VI. References cited</td>
<td>24</td>
</tr>
<tr>
<td>VII. Scientists contributing to this report</td>
<td>34</td>
</tr>
</tbody>
</table>
I. INTRODUCTION

This report concerns the health aspects of using vitamin D₂ and vitamin D₃ 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*. 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, announcement was made in the Federal Register on June 16, 1978 (43 FR 26132) 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 vitamin D₂ and vitamin D₃ as food ingredients. One request was received but subsequently withdrawn. However, since withdrawal of the request occurred after public announcement of the hearing in the Federal Register on September 15, 1978 (43 FR 41277), a hearing was held as announced on September 25, 1978; details are given on page 35.

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 (2) [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 (2) recognizes further [21 CFR 170.30] that it is impossible to provide assurance that any substance is absolutely safe for human consumption.

*The document (PB 234-901/7) is 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 evaluations 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 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 vitamin D₂ and vitamin D₃ 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

Vitamin D is historically the antirachitic vitamin and currently it is considered both a vitamin and a hormone. Vitamin D activity is exhibited by several compounds; some occur naturally and some are synthetic. The principal compounds, and the only ones considered in this report, are vitamin D$_2$ (ergocalciferol) and vitamin D$_3$ (cholecalciferol). Ergocalciferol is 3 (β)-hydroxy-9, 10-seco-6("cis")-6, 7-s-trans-ergosta-5, 7-10(19)22-tetraene. It is produced by irradiation of ergosterol isolated from yeast and related fungi. Cholecalciferol is 3(β)-hydroxy-9, 10-seco-6("cis")-6, 7-s-trans-cholesta-5, 7-10(19)-triene. It is produced naturally through the sunlight activation of 7-dehydrocholesterol in the skin (3).

Antirachitic activity can be elicited by exposure of the skin to ultraviolet irradiation, by provitamin D compounds, or foods and other materials containing such compounds. The natural food sources of vitamin D are basically limited to fish and fish oils, eggs, liver, butter, and milk (3, 4). Milk is a relatively poor source of vitamin D unless it is fortified (4).

Vitamin D activity is measured by: (a) bioassay in suitably conditioned rats where the reference standard is the biological activity shown by 0.025 μg of vitamin D$_2$. (This quantity of the vitamin is considered one International Unit (IU); making 1 μg of vitamin D$_3$ equivalent to 40 IU; (b) chemical analysis in which the reference standards are known forms of the vitamin (3). Bioassays measure total vitamin D activity and do not distinguish between the chemical forms. Chemical analyses can measure vitamin D$_2$, vitamin D$_3$, and other forms specifically (5).

Federal Regulations (2) list vitamin D$_2$ [21 CFR 182.5950] and vitamin D$_3$ [21 CFR 182.5953] as GRAS nutrients and/or dietary supplements. Only these two substances, vitamin D$_2$ and vitamin D$_3$, are added commercially to food as vitamin D supplements.

Vitamin D$_2$ occurs as white, odorless crystals, melting at 115 to 118°C, with a specific rotation, [$\alpha$]$_D^{25}$, of +103 to +106°; it is insoluble in water but soluble in alcohol, chloroform, ether, and fatty oils. Limitations are specified for ergosterol and reducing substances. Vitamin D$_3$ occurs as white, odorless crystals, melting between 84 and 88°C with a specific rotation, [$\alpha$]$_D^{25}$, of +105 to +112°; it is water-insoluble but soluble in alcohol, chloroform, and fatty oils (6).

Some quantitative studies and calculations have been made of the synthesis of vitamin D by the action of sunlight on sterols present in the skin.
Loomis (7) calculated that exposure of 20 cm² of white human skin to sunlight can result in the synthesis of up to 400 IU of vitamin D in about 3 hours. Thomson (8) found that the average transmission of solar ultraviolet radiation (about 3000 to 4000 Å wavelength) through the stratum corneum of 22 Europeans was 64 percent whereas the average for 29 Africans was only 18 percent.

With respect to daily requirements of vitamin D the Committee on Nutrition of the American Academy of Pediatrics (9) has concluded that 400 IU per day provides an adequate margin to protect substantially all growing individuals from detectable incidence of vitamin D deficiency. The Food and Nutrition Board of the National Research Council (4) has concluded that there is no information concerning the precise requirements of vitamin D in older children and adults. They observed that 100 IU per day prevents rickets and ensures adequate calcium absorption and bone mineralization in full-term infants but recommend a daily allowance of 300 to 400 IU which seems to provide better calcium absorption and some increase in growth. They believe that an intake of 300 to 400 IU of vitamin D per day is advisable for pregnant and lactating women, children, and adolescents. The Board also concluded that no dietary requirement of vitamin D for normal healthy adults is necessary since the requirement is satisfied by sunlight irradiation; only persons whose activities limit their exposure to sunlight need have a dietary source of vitamin D. This conclusion is supported by the studies referred to above where 20 cm² of exposed, white, human skin was found to synthesize up to 400 IU in 3 hours (7). On this basis 300 to 400 IU of vitamin D per day could readily be derived from this source under most conditions.

The FAO/WHO Handbook on human nutritional requirements has recommended daily vitamin D intakes of 10 µg (400 IU) for ages up to 7, 10 µg for pregnant and lactating women, and 2.5 µg for all others (10).
III. CONSUMER EXPOSURE DATA

The assessment of exposure to vitamin D is subject to all the difficulties associated with estimating intakes of other nutrients. It is further complicated because of the unique capacity of sunlight to form vitamin D from 7-dehydrocholesterol in the skin. Such conversion is affected by an array of factors including the length and intensity of solar radiation and the area of body surface exposed (3).

A subcommittee of the National Research Council surveyed manufacturers by questionnaire regarding the level of addition of vitamin D₂ and vitamin D₃ to foods in 1970 and the total quantities used in that year in food supplementation (11). Vitamin D₂ was added to baked goods, breakfast cereals, pastas and rice dishes, fats and oils, milk and milk products, meat and poultry products, sauces, nonalcoholic beverages, dairy product analogs, and baby formulas; vitamin D₃ was added to fats and oils, milk and milk products, and baby formulas. In reviewing these data and after discussions with the NRC staff, the Select Committee has concluded that there were inconsistencies in the responses to the survey questionnaire, leading to errors in the mean levels of addition and overestimates of the daily intakes calculated from them. For these reasons the Select Committee has sought other means for estimating daily intakes.

In 1963 the Committee on Nutrition of the American Academy of Pediatrics (9), attempted to estimate the vitamin D intake of individuals in the United States and Canada at ages ranging from 6 months to 8 years. The possible content of vitamin D in common vitamin D-supplemented foods was computed from the manufacturer’s label claims. Hypothetical menus were constructed and, using the values for the reported vitamin D content of the hypothetical meals, two diets were devised for each of three age groups. One diet was selected to represent an arbitrarily average situation, the other to represent a situation in which the intake was clearly, but realistically high. The calculations took into account the amount of vitamin D added as a supplement. It was apparent that average 6-month-old babies were receiving about 400 IU from food and 400 from supplements, making the total estimated daily intake 800 IU (about 160 IU per kg body weight). For those representing the more generously supplemented babies it was calculated that the daily intake of vitamin D was approximately 1400 IU (about 280 IU per kg). On the same basis it was found that 3-year-old children in the average category were receiving approximately 600 IU (about 40 IU per kg) daily and those getting larger vitamin D supplements probably averaged 2600 IU (about 170 IU per kg); for 8-year-olds the figures were 800 IU and 2900 IU (about 30 to 100 IU per kg) daily, respectively.
In 1967 Dale and Lowenberg (12) surveyed the vitamin D intakes of six groups of 25 subjects from neonates to 17-year-olds. They found that the use of vitamin preparations in addition to vitamin D-fortified foods resulted in daily vitamin D intake in infants ranging from 130 to 914 IU (average, 462 IU). In the preschool group the range of daily intakes was 21 to 1,137 IU (average, 660 IU). For all 150 subjects the range was 21 to 1800 IU (average, 547 IU). In most instances the principal source of vitamin D was fortified milk.

In 1970 a Joint WHO/FAO Expert Group (13) reported that intake data in populations were virtually non-existent except for a British estimate of 116 to 133 IU per person per day covering both rural and urban populations.

In 1971 Lumb et al. (14) considered relationships between blood serum vitamin D levels and exposure to vitamin D. They cited a preliminary dietary survey made in the northwest of England which indicated that many healthy people ingested as little as 30 to 60 IU per day, and that daily intakes above 150 IU were unusual. These survey findings were compatible with the low levels of vitamin D activity assayed in the serum of some of their adult subjects.

Based on such data, the Select Committee concludes that daily vitamin D intake via food and supplements in the United States, including that added to supplement foods, is no more than 1600 IU (about 300 IU per kg) for infants, 2600 IU (about 170 IU per kg) for 3-year-olds, and 2900 IU (100 IU per kg) for 8-year-olds. How much of these totals is due to the vitamin D₂ and/or vitamin D₃ added to foods is not precisely known but, based on the reported vitamin D content of some unfortified foods (15) and the consist of the daily intake reported by the Committee on Nutrition of the American Academy of Pediatrics (9), there is reason to believe that added vitamin D in foods and supplements represents the major part of total dietary intake. However, it should be noted, as pointed out by Kovacs (16) that fortification of milk is not mandatory and that information is not available concerning the amount of whole milk and low fat milks that are currently being fortified with vitamin D or the relative amounts of each that are consumed. Since fortified milk is a major source of dietary vitamin D, and since per capita consumption of low fat milks relative to whole milk has increased considerably in recent years, it would be desirable to obtain new data upon which to base better estimates of current daily intakes of vitamin D from dietary sources.
Absorption and excretion

All known forms of vitamin D are absorbed with food fats. Thus, essentially all factors affecting the absorption of fat in the alimentary tract affect the absorption of vitamin D. Absorption occurs in the jejunum and/or ileum, with most of the vitamin in the chylomicrons of the lymphatic system. Bile is essential for absorption of vitamin D and it also seems to be the major pathway of excretion of vitamin $D_2$ and vitamin $D_3$ after they have been metabolized to certain hydroxy derivatives (17).

Absorbed vitamin D circulates in the blood (14). Metabolites of vitamin D are formed in the liver and in the kidney; movement from the plasma chylomicrons and lipoproteins appears to occur in the liver by transfer to an $\alpha_1$-globulin fraction which acts as a carrier (17, 18). Kodicek (19) has reviewed evidence to show that vitamin D is absorbed through the skin after topical application.

Metabolism

As early as 1934 Waddell (20) found that crude cholesterol preparations contained a provitamin D constituent which, after irradiation, was more potent against rickets in chicks and hence different from that obtained by irradiating ergosterol. Since that time the multiple nature of vitamin D has been established, and, in the last decade, much knowledge has accumulated concerning the metabolism of the vitamins D and their modes of action (3, 17, 21, 22). It is apparent that vitamin $D_2$ and vitamin $D_3$ require metabolic conversion before they can function as the vitamin or hormone. The major developments involve several hydroxylated metabolites of vitamin D, with most of the attention focused on the metabolism of vitamin $D_3$. After transport to the liver, a hydroxylase system in the microsomal component effects hydroxylation on carbon atom-25. Some evidence suggests that this process is feedback regulated by the concentration of the 25-OH-$D_3$ metabolite in the liver. A specific protein transports the 25-OH-$D_3$ to the kidney where further enzymatic hydroxylation occurs to yield 1,25-(OH)$_2$-$D_3$ or 24,25-(OH)$_2$-$D_3$, as determined by physiological circumstances. The 1,25-(OH)$_2$-$D_3$ is 10 to 15 times more potent than vitamin $D_3$ when administered parenterally. It is the most potent form of vitamin D known in effecting intestinal calcium absorption, bone calcium mobilization, and elevation of serum phosphate. In response to hypocalcemia, the parathyroid glands secrete parathyroid hormone. This triggers the synthesis of 1,25-(OH)$_2$-$D_3$, which with the parathyroid hormone, functions in the mobilization of calcium from bone and without the parathyroid hormone, effects calcium absorption in the intestine. Such processes involve a dynamic balance in which elevated levels of serum calcium suppress secretion of parathyroid
hormone, consequently decreasing the synthesis of 1,25-(OH)$_2$D$_3$. Thus, the latter can be regarded as a calcium metabolizing hormone as well as a vitamin.

Owing to the multi-step nature of the processes leading to the metabolic formation of 1,25-(OH)$_2$D$_3$, an impairment in any step can be presumed to be harmful. Diseases which may be attributed to insufficient vitamin D may in fact be due to a metabolic block in the formation of 1,25-(OH)$_2$D$_3$. Bone disease associated with renal failure may involve an impairment in the 1-hydroxylation process. Also, hypoparathyroidism can be linked with an impairment of 1,25-(OH)$_2$D$_3$ synthesis. This is indicated by the effectiveness of this vitamin D$_3$ metabolite in parathyroid hormone deficiency as produced through thyroparathyroidectomies in rats and occurring spontaneously in hypoparathyroid patients (3). For example, DeLuca (23) has stated, that treatment with 1 μg per day of 1α, 25-(OH)$_2$D$_3$ is entirely successful while 2,000 μg of vitamin D$_3$ are required to be effective in treating the disease.

Minimal responsiveness to vitamin D is generally referred to as vitamin D dependency, an inherited postnatal syndrome (24). Alleviation of the syndrome may occur if there is continued therapy with large amounts (1.25 mg or about 50,000 IU) of vitamin D per day (25). The occurrence of this hereditary trait is low but the incidence appears to be higher than once suspected (26). The newer knowledge of vitamin D is having marked effects in the interpretation of treatment of the vitamin D-dependent, as well as normal individuals, with massive doses of vitamin D (17).

Progress in the study of vitamin D metabolites and the interrelationship of vitamin D to the parathyroids has been comprehensively reviewed by DeLuca (23), Omdahl and DeLuca (27), Hay (28) and Harrison (29).

**Animal toxicity studies**

The extensive literature on the effects of vitamin D administration in many species has focused on crystalline vitamin D$_2$ and vitamin D$_3$, but also includes studies on the effects of related sterols having vitamin D activity, as well as rich sources of vitamin D, such as high potency fish liver oils (1).

The LD$_{50}$ of vitamin D$_3$ sulfate given intraperitoneally to mice was found to be about 2,500,000 IU per kg (30).

Minimal dosage for production of generalized calcinosis by intramuscular injection of vitamin D$_3$ in male rabbits was 500,000 to 600,000 IU given in 3 equal doses at intervals of 2 days. Signs of generalized calcinosis appeared after 8 days (31); dosage was about 45,000 to 50,000 IU per kg per day.
Calcinosis increased in severity on such a regimen if injections were extended to 3 weeks. Anorexia, loss of weight and death occurred within about 6 weeks. Vitamin D₃ given subcutaneously daily for 3 consecutive days to rabbits at a level of 5,000 IU per kg per day, showed increased calcification of dentine but no side-effects (32). Administration of 10,000 IU per kg per day by the same procedure led to anorexia, weight loss and death in five to seven days. By contrast a dose of 800,000 IU of vitamin D₃ per kg daily, given subcutaneously, elicited no side-reactions. Vitamin D₃ in cottonseed oil was administered intramuscularly every other day for 30 days to 8 adult female rabbits beginning the day after observed copulation (33). Total dose over the 30 day period was 1.5 million IU (about 50,000 IU per kg) on each day of injection. No differences were observed between these animals and controls receiving no injected vitamin D₃ except for higher vitamin D levels in the blood of the former. Pregnant females given a total dose of 2.5 million IU (about 85,000 IU per kg per day) or more over the 30 day period died 65 days after the first injection.

In an early study performed before an IU for vitamin D had been established, groups of weanling rats were fed a stock diet containing enough irradiated yeast to supply 40 times the rickets-curate dose of vitamin D (34). Irradiated ergosterol was added to this diet in amounts necessary to provide vitamin D activity equivalent to 50, 1,000, 10,000, or 100,000 times the curative level. Amounts as high as 10,000 times the daily curative dose over a period of 6 months had no effect on growth and body functions. At levels 100,000 times the daily curative dose toxic effects were observed, including anorexia, labored breathing, net loss of calcium and phosphorus from the body, hypercalcemia, skeletal decalcification, and renal calcification. In more detailed studies Harris et al. (35) fed weanling rats for 20 days on a basal diet containing irradiated ergosterol (vitamin D₃) or tuna liver oil (vitamin D₂) at a dose of about 500,000 IU per kg daily. The authors observed that this dose was at least 5,000 times the therapeutic dose for rats. There was weight loss, and calcification occurred in the kidneys, stomachs, aortas, hearts, and lungs of rats receiving either source of vitamin D. As measured by such criteria, irradiated ergosterol was more toxic than tuna liver oil.

In a study of only one source of vitamin D (electrically activated capro- ized ergosterol), rats fed up to 20,000 IU per kg daily for varying periods of time ranging from 100 days to 190 days showed no histological changes in the heart, lungs, liver, spleen, pancreas, stomach, adrenal glands, kidneys, aorta, or brain (36). Likewise, no roentgenographic or other abnormalities suggestive of toxic effects were noted for this preparation at the dose employed. Other investigators (37) fed rats a stock diet in which the activated ergosterol (vitamin D₃) was incorporated daily during the last 2 weeks of a total study period of 4 weeks. The daily dose of vitamin D was 60,000 to 100,000 IU per kg body weight. Based on comparisons with a control group, there were no signs of harmful effects on growth rate, bone and tooth formation, tooth eruption rates, estrus cycle, possible occurrence of tooth pulp stones, or
general appearance and behavior.

There is a suggestion in the studies of Zemplenyi and Mrhova (38) that adult male rats, fed a basal diet containing 0.7 percent cod liver oil, undergo vascular enzyme changes when they are fed, in addition, about 75,000 IU vitamin D$_2$ in oil daily for periods of 5 to 9 days. In the aorta there was an increase in the activity of phosphomonoesterase I and II and of 5'-nucleotidase, whereas the activity of carboxylic esterase decreased slightly. The investigators interpreted the changes in the phosphomonoesterases and nucleotidase as reflecting an increase in vascular connective tissue activity. The authors speculated that the slight change in carboxylic esterase might be related to an increase in abnormal polysaccharides or calcium salts in the aorta.

Cruess and Clark (39) in well-controlled experiments, fed male rats daily 150,000 IU vitamin D$_2$ per kg body weight dissolved in sesame oil for up to 24 days. Growth was impaired, bone ash was decreased and the organic fraction of the bones (including all the phospholipids examined) was increased. On the basis of $^{32}$P uptake measurements it was apparent that hypervitaminosis D$_2$ increased the synthesis of phospholipids.

Hass et al. (31) observed histologically the distribution and evolution of lesions of the vascular system in male rabbits dosed intramuscularly with 43,000 to 260,000 IU vitamin D$_2$ per kg body weight daily, twice weekly or thrice weekly for periods as long as 6 to 8 weeks. The amount and distribution of abnormal calcium deposits in the soft tissues increased with increasing duration and size of the vitamin D dose. The total minimal dose for production of significant generalized calcinosis was 500,000 to 600,000 IU administered over a period of 3 weeks, but the authors note that interpretation of their results was complicated by intermittent chronic purulent pyelonephritis and hepatic coccidiosis in both experimental and control animals. Calcium was most conspicuously distributed in the aorta and its main branches, and somewhat less so in the kidneys. It was concluded that administration of vitamin D in amounts just sufficient to produce pathologic changes in 2 or 3 weeks led to mineralization of certain tissues which do not normally calcify.

Steck et al. (40) fed adult dogs vitamin D$_2$ in corn oil in daily dosages of 15,000 to 500,000 IU per kg body weight. In amounts greater than 50,000 IU per kg daily the average survival time was about 12 days; with 20,000 IU or less per kg daily there was survival for indefinite periods without signs of intoxication. While weight loss, hypercalcemia, and kidney calcification were prominent in animals receiving the higher doses, these signs were absent in animals receiving 20,000 IU or less per kg body weight. Hendricks et al. (41) concluded that the cumulative effects on tissue calcification in dogs of capsule doses of about 5,000 IU vitamin D per kg body weight per day over a period of 10 months were not as severe as a single dose of about 50,000 IU per kg body weight.
Kent et al. (42) studied a colony of 558 monkeys (Mucaca mulatta) 3 to 9 years old, weighing 2.5 to 10 kg, that were inadvertently fed excessive amounts of calcium, phosphorus, and vitamin D (kind not stated) for about 3 months. During this period each animal received daily approximately 162,000 IU (about 16,000 to 65,000 IU per kg) vitamin D, and 3.5 g of calcium and 2.9 g of phosphorus. There was weight loss and an increase in the incidence of upper respiratory tract infections and diarrhea. Calcium and iron deposits occurred in the kidneys, salivary glands, and lungs and were found in the aortas of 12 of 34 animals dying between the 55th and the 140th day. About 1 month after termination of the dietary excesses, the surviving animals appeared to be in good health and monkeys sacrificed after 1 year showed few lesions that were considered related to hypervitaminosis D.

Comparative studies on the toxicity of different sources of vitamin D fed in excessive amounts to dogs indicated that a commercial source of vitamin D₂ had a far more rapid and severe toxic effect (calcification of soft tissues) than did equal dosage of vitamin D administered as tuna liver oil. Doses were approximately 10,000 IU per kg per day over a period of 8 to 10 months (41). Jung (43) using crystalline vitamins D₂ and D₃ and irradiated ergosterol in oral doses up to 300,000 IU per kg per day in rats, found no difference in their toxicity (weight loss) but similar doses of 7-dehydrocholesterol were found to be 2 to 3 times more toxic than any of the other sources. Mrazek et al. (44) in comparing the chronic toxicity of vitamin D₂, and vitamin D₃ and activated ergosterol in adult rats, intubated 75,000 IU per kg per day until all of the animals died. The average survival time of rats receiving vitamin D₂ was about 43 days, vitamin D₃ about 22 days, and activated ergosterol about 70 days. McChesney (45) found that orally, vitamin D₃ is about 55 percent more toxic and dihydrotachysterol about 260 percent more toxic than vitamin D₂ in the rat. He concluded that the relative toxicities of vitamin D₂ and D₃ correlate well with their hypercalcemic effects. Hunt et al. (46) using daily oral doses of 50,000, 100,000 and 200,000 IU of vitamin D₂ or vitamin D₃ in rhesus monkeys found that vitamin D₂ was significantly more toxic than vitamin D₃ in this species. Animals given vitamin D₃ at all dose levels died within 160 days. Those receiving vitamin D₂ survived, developed hypercalcemia, but showed no soft tissue mineralization after sacrifice.

In a long-term study by Bills and Wirick (47) on the effects of overdoses of vitamin D, done prior to the establishment of the International Unit, and employing approximately 1200 rats including second and third generations, it was concluded that 0.25 percent activated ergosterol added to the diet (equivalent to 100 times the minimum antirachitic level) caused no observable effect on appearance, growth, reproduction, or resistance to respiratory infections. One thousand times the antirachitic dose was found to be "perceptibly harmful," 4000 times "definitely injurious," and 40,000 times "strongly toxic."
Human toxicity studies

Of a large number of reports on the effects of excessive vitamin D intake in humans, several are noteworthy.

Steck et al. (40) summarized their observations on 773 subjects ranging in age from 17 to 76 who were given supplemental doses upward of 3000 IU vitamin D (presumably as activated ergosterol) per kg per day for 7 days to 5 years. They concluded that the shortest period of administration producing toxicity (weight loss, increased calcium excretion) in the group receiving 3000 to 5000 IU per kg per day was 87 days. In the group receiving 6000 to 7000 IU per kg per day the shortest period for toxicity to occur was 60 days. Of the 773 subjects, about 16 percent showed toxic signs at levels up to 25,000 IU per kg per day. The adverse effects were found to be reversible even at the highest level when dosing was discontinued.

Cogan, et al. (48) described five patients who had taken doses of vitamin D varying from 100,000 to 500,000 IU (about 1,700 to 8,300 IU per kg) daily over 2.5 months to 5 years for treatment of rheumatoid arthritis or pruritus. All patients showed hypercalcemia, band keratopathy, and evidence of renal insufficiency as indicated by elevated blood nonprotein nitrogen and inorganic phosphorus. There was no bone decalcification or elevation in serum alkaline phosphatase.

Howard and Meyer (49) reviewed the cases of 11 adult patients age 33 to 68, who had been given daily doses of vitamin D2 (activated ergosterol) ranging from 150,000 to 600,000 IU (about 2,500 to 10,000 IU per kg daily) as a therapeutic measure against arthritis. Fatigue, weight loss, and anorexia were observed in 2 to 18 months. The outstanding clinical signs were impairment of renal function and degenerative lesions with calcification such as band keratitis. In all cases the blood nonprotein nitrogen and serum calcium were elevated.

Chaplin et al. (50) reviewed 111 cases of vitamin D intoxication appearing in the literature and described 7 additional cases under their observation. All seven had been taking 50,000 to 300,000 IU (about 1,000 to 6,000 IU per kg) of vitamin D daily for periods ranging from 3 weeks to 6 years. Band keratitis, calcium-containing periarticular cysts in the vicinity of bursae, and hypercalcemia were among the characteristic findings.

DeLuca and Cozzi (51) reported on 12 infants, ages 7 to 30 months, intoxicated by doses of 48,000 to 200,000 IU (about 10,000 to 40,000 IU per kg) daily for 30 to 50 days. The signs and symptoms observed included anorexia, restlessness, pallor, hypercalcemia, lowered alkaline reserve, and renal insufficiency.
Debré (52) found in a clinical study of 21 cases of vitamin D overdosing in children, aged 16 months to 7 years, that a total intake of 11 to 18 million IU (time period not stated) was fatal in two instances. Patients receiving total intakes of 3 to 6 million IU (time period not stated) experienced anorexia, vomiting, thirst, headache, and joint pain. These symptoms disappeared within 10 days after vitamin D dosing was stopped.

Other effects associated with vitamin D intakes of 1,000 to 3,000 IU per kg daily for periods up to several years include confusion, lethargy, and behavioral and neuropsychiatric disturbances (53), manic-depressive psychosis (54), mental depression, general malaise, and headache (55), and anemia (56).

There appear to be no recorded cases of vitamin D toxicity from over exposure to sunlight and no reported studies of the relative toxicity of vitamin D₂ and vitamin D₃ in humans (57).

**Linear growth of infants**

The conclusion of Stearns (58) that "the critical upper safe level for continuous intake of vitamin D must be somewhere between 800 and 1500 units daily" raises a question about adverse effects of rather modest intakes of vitamin D and therefore merits careful evaluation. The control group employed in the comparisons consisted of 36 male infants observed by Stearns et al. (59) in a metabolic ward and fed 340 to 400 IU of vitamin D daily during all or part of the first year of life. These infants received excellent care and presumably had little exposure to infection. Although it is acceptable to compare performance of this group with that of other groups studied under similar circumstances, growth comparisons of small groups of subjects are likely to be misleading and must be examined in detail. Unfortunately, this is not possible with respect to the linear growth of the infants studied by Stearns et al. (59). The number of subjects available at each age was not stated and the presentations concerned semilongitudinal size data rather than incremental data. In most instances, the mean body length at various ages was presented without statistical summary of variability (e.g., confidence intervals), and statistical analysis of the differences between groups was not included.

Stearns stated, "It is emphasized that the slowing of growth noted with excessive vitamin D dosage does not appear in most infants until about five or even six months of age" (58). She stated that 22 infants studied by Jeans and Stearns (60) received 1800 to 4500 IU of vitamin D daily but it is not clear how many of these infants were studied for more than 6 months. Detailed data have been published concerning only 9 of these 22 infants and 4 of the 9 received doses of vitamin D of 1800 IU or more daily only until 15 to 20 weeks of age. Thus, evaluation of the data must rest on observations of 5 infants. Charts presenting body length versus age suggest that one infant
(subject G) may have had some slowing of linear growth after 26 weeks of age. Another infant (subject S) may have demonstrated some slowing after 26 weeks of age but initiation of more rapid growth appeared to precede the decrease in dosage of vitamin D. Growth of the remaining three infants did not appear remarkable. The Select Committee considers these data inadequate to support a conclusion that moderate overdosage of vitamin D (1800 to 4500 IU daily) interferes with linear growth.

Equally unimpressive as support for Stearns' conclusion are her comparisons of linear growth of the 36 infants given 350 to 400 IU of vitamin D daily while living in the metabolic unit, with data on the linear growth of larger groups of infants living at home and studied by other investigators (61, 62). Because of the great differences in environment and probably in aspects of nutritional management other than intake of vitamin D, such comparisons seem inappropriate.

Fomon et al. (63) studied three groups of male infants; one group received 350 to 550 IU vitamin D daily; a second group 1380 to 2170 IU daily; a third group 300 IU daily. The latter group was breastfed and the other two groups received evaporated milk formulas. During a test period of 168 days the rates of growth in length and weight and the serum concentration of calcium were similar in the three groups. This study failed to provide evidence that the level of overdosage of vitamin D interferes with the growth and well-being of normal infants.

**Idiopathic hypercalcemia**

Unexplained hypercalcemia of infants was first described in 1952 (64, 65) and circumstantial evidence implicating vitamin D in its pathogenesis was presented. Several reviews (66-69) have helped to provide perspective on the relation between intake of vitamin D and the disorder known as "idiopathic hypercalcemia (of infancy) or infantile hypercalcemia."

The disorder is generally classified as mild or severe, although some cases cannot be easily assigned to one of these two categories because of intermediate manifestations. The best available data on incidence are from the United Kingdom where in 1960-1961 there were 35 cases per year with about 785,000 births, giving an incidence of 1 case in 20,000 live births (67). Because approximately 8 percent of the British cases were classified as severe, the incidence may be estimated to be 1 severe case for every 275,000 births.

Patients with the mild form demonstrate hypercalcemia and, by 3 to 7 months of age, generally demonstrate mild growth retardation. Although there are some exceptions, long-term prognosis is generally excellent. Patients with the severe form demonstrate hypercalcemia, failure to thrive, a characteristic "elfin" facies, impairment of renal function, severe mental retardation.
dense mineralization of the base of the skull and metaphyses of the long bones, and not uncommonly supravalvular aortic stenosis or peripheral pulmonary artery stenoses (66).

In the mid 1950s it was estimated (69) that a substantial number of normal infants in Britain might receive as much as 4000 IU of vitamin D per day by ingesting nationally subsidized or commercially prepared milk powders, infant cereals and one of the commonly employed vitamin D supplements. According to the American Academy of Pediatrics (66), in 1956 the British Paediatric Association, through a survey of its members, reported that in a 30-month period from 1953-55, a total of 216 cases of infantile hypercalcemia were encountered (about 7.2 cases per month). Because of the possibility that this was related to moderate overdosage of vitamin D, drastic reductions were advised in the amounts of vitamin D added to certain foods. By 1957, manufacturers had already reduced the extent of vitamin D supplementation. By 1959, it was believed that the old products must nearly have disappeared from the retailers (68) and another survey of the members of the British Paediatric Association was carried out. A negligible decrease to about 6.8 cases per month was reported (69). However, the British Ministry of Health noted that certain vitamin supplements still provided 800 IU of vitamin D daily and steps were taken to reduce the dosage or provide a warning on the label. A third survey of the members of the British Paediatric Association was conducted in 1960-61 and demonstrated a substantial fall in prevalence of idiopathic hypercalcemia to about 3.0 cases per month (69). The cautious conclusion of the British Paediatric Association (69) was as follows:

"...This is probably a real decrease in the prevalence of hypercalcemia, because the methods of inquiry and the number of responding paediatricians were comparable and the impression of most clinicians supports the quantitative data. The decrease might in fact be greater than appears if the diagnosis of hypercalcemia has become progressively more accurate since 1953. The marked decline in hypercalcemia does not correspond in time with the major reduction in vitamin D allowances which took place in 1957-58, but followed it after an interval of two to three years."

Other authors (70) seemed somewhat more convinced of the relation between reduction in intake of vitamin D and the decreased incidence of idiopathic hypercalcemia. It is to be noted that the incidence figures pertain at least primarily to the mild form of idiopathic hypercalcemia. Whether there was a decrease in incidence of the severe form in Britain in the late 1950s is unknown.
Several additional bits of information suggest the need for caution in assigning vitamin D a causative role in etiology of the mild form of idiopathic hypercalcemia. Of the 50 cases (which apparently included 4 cases of the severe form) identified by the 1960–61 survey of the British Paediatric Association, 28 percent were stated to have received no vitamin D supplements before the onset of the disease (69). Serum vitamin D concentrations have been found to be in the normal range in mildly affected infants and in severely affected infants have been markedly increased in some patients and normal in others (67). Some but not all mildly affected infants demonstrate an exacerbation of hypercalcemia when challenged with vitamin D (67).

There is no evidence that women whose offspring were severely affected in utero had ingested more than 400 to 800 IU of vitamin D daily during pregnancy (67). Furthermore, infants born to women with hyperparathyroidism do not demonstrate any of the manifestations associated with the severe form of idiopathic hypercalcemia (71). As summarized by the Committee on Nutrition of the American Academy of Pediatrics (66), if vitamin D plays a role in the intrauterine development of the severe form of infantile hypercalcemia, it must do so either by placental transfer of the vitamin from the mother to the excessively vitamin D-sensitive fetus or, alternatively, by producing a response in the excessively-sensitive mother which is deleterious to the fetus.

Vascular effects

Dalderup et al. (72) and Knox (73) have proposed an association between excessive vitamin D intake and death from ischemic heart disease. Linden (74) and Westlund (75) reported an association between renal calculi and coronary heart disease. Lindén (76) studied 341 men and women in Norway with disorders which qualified them for disability pensions. These included 150 persons with myocardial infarction. The disabilities included, besides myocardial infarction, angina pectoris and degenerative joint diseases. In the study were 341 controls randomly selected in the same area and of the same sex and age distribution, but without qualifying for disability pensions. Both men and women with myocardial infarction were consuming somewhat larger amounts of vitamin D (more than 1250 IU per person per day) than any of the other groups (approximately 900 IU per person per day). There was no difference between the controls and the subjects with angina pectoris and degenerative joint diseases. The data give some support to Lindén's hypothesis that long-term ingestion of excesses of vitamin D may be a factor in the occurrence of myocardial infarction.

Kummerow et al. (77) reported that the abdominal aorta from weanling swine fed 100,000 IU vitamin D₃ per pound of feed for 5 weeks had localized collections of fibrous and amorphous extracellular material. Smooth muscle cells appeared atypical. This did not occur in the controls on the same diet which contained 650 IU vitamin D₃ per pound of ration. The vitamin D content
of the tissues of weanling swine given the large vitamin D₃ supplement for 6 weeks was several orders of magnitude higher than in the unsupplemented controls. The blood serum, muscle, fat, and liver from normal human subjects assayed for higher levels of vitamin D than these tissues from unsupplemented swine that had been fed a regular corn and soybean commercial ration (78). There appear to be no studies in which an excess of vitamin D has been given in somewhat smaller doses and for longer periods of time.

Pregnancy and neonatal effects

Massive doses of vitamin D to rats have long been known to affect the estrus cycle, fertilization, and course of pregnancy. In a study of Ornay et al. (79) vitamin D₂ doses of the order of 4,000, 20,000, and 40,000 IU (about 10,000, 50,000, and 100,000 IU per kg, respectively) were administered daily by intubation to pregnant and nonpregnant rats. Four thousand and 20,000 units administered from the 9th day of pregnancy had no apparent deleterious influence on the fetal development. At a dose of 40,000 IU the placentas, fetuses, and bones were all small, and the progeny were nonviable. The results suggest that vitamin D₂ or a metabolite passes through the placental barrier.

In experiments with rabbits given total dosages of 1.5, 2.5, 3.5, or 4.5 million IU (about 50,000 to 150,000 IU per kg body weight on each day of intramuscular injection) of vitamin D₂ throughout the term of pregnancy (30 days) it was shown by Friedman and Roberts (33) that the vitamin, or a metabolite, crossed the placenta. The blood levels of antirachitic substance in the mothers given vitamin D and their offspring were seven and nine times greater than in the control mothers and offspring, respectively. Also, the serum calcium levels in the offspring whose mothers received vitamin D were significantly higher than those of controls.

Nonfamilial, congenital supravalvular aortic stenosis syndrome is a concomitant of idiopathic infantile hypercalcemia (80). In the study of pregnant rabbits given intramuscular doses of about 25,000 IU vitamin D₂ per kg body weight throughout pregnancy, 14 abnormal aortas were noted in the 34 offspring. Six additional offspring at three months showed generalized vitamin D vasculotoxicity, without supravalvular narrowing of the aorta, of an advanced type commonly seen in the adult animal given large doses of the vitamin (35). None of the control animals showed any abnormalities of the aorta. Thus, this study strongly suggests that large doses of vitamin D during pregnancy have an effect on the fetus and the excessive vitamin, or a metabolite, may be responsible for supravalvular aortic stenosis.

In related studies by Friedman (81) and Friedman and Mills (82) in rabbits, relationships were found between vitamin D excess in pregnancy and certain craniofacial and dental anomalies of the supravalvular aortic stenosis syndrome. Fifteen pregnant rabbits were given divided doses of vitamin D₂
intramuscularly in cottonseed oil every other day, starting on the second
day after insemination and continuing until delivery. The total dose admin-
istered was 750,000 IU, an average of approximately 27,000 IU (about 13,000
IU per kg body weight) per day. The offspring from the vitamin D-treated
mothers had hypoplasia of the mandible, congenital absence of teeth, microdontia,
and enamel hypoplasia. The most marked functional accompaniment of these
abnormalities was severe malocclusion of the teeth. Many of the test animals
had peculiar facies, premature closure of the cranial bones, strabismus,
odd-shaped ears, and low birth weight.

In the interpretation of experiments based on the use of massive oral
doses (e.g. 50,000-100,000 IU per kg daily to rats during the last 9 days of
pregnancy) of vitamin D it should be recognized that such amounts reportedly
not only damage the placenta but also alter the normal physiologic transfer
mechanisms of the placental barrier (83). However, such effects do not
invalidate the experiments as a whole. Haddad et al. (89) have shown that the
placental transfer for vitamin D₃ and its active hydroxylated metabolite proceeds
at comparable rates. Also, the data suggest that vitamin D₃ metabolites cross
the placenta rapidly and/or are produced by the fetuses. The findings leave
room for the possibility that the fetus is capable of transforming vitamin D₃
from the mother to the metabolite(s) observed in the fetal tissue. The data
support the suggestion made in 1967 that idiopathic hypercalcemia and supra-
valvular aortic stenosis of infants might be the consequence of a vitamin D
metabolite produced in maternal or placental tissues during pregnancy (85).

The variability between individuals and genetic strains in the response
to maternal excesses of vitamin D has been shown in various studies. The
findings of Ornoy et al. (86) illustrate such variability. Wistar strain rats
were given 40,000 IU (about 200,000 IU per kg) ergocalciferol in olive oil
daily by intubation from the tenth to twenty-first day of pregnancy. Charles
River rats (a more susceptible strain) were similarly dosed with 40,000
IU and a separate group was given 20,000 IU of the vitamin. The young of all
Charles River rats died even at the smaller dose level. Seven of the litters
of 14 Wistar rats given 40,000 IU of vitamin D per day survived beyond the
first postnatal day. The surviving offspring showed no gross malformation
at birth but after a few days skeletal deformities developed. It was concluded
that the postnatal effects at these high dosage levels were not due to persist-
tence of an excess of vitamin D in the tissues or through the milk, but resulted
from a fundamental teratogenic effect on the fetal osteogenic tissues which
became manifest as the bone developed. In the same laboratory (87) it was
reported that toward the end of pregnancy in Wistar rats receiving oral doses
of 20,000 to 40,000 IU of vitamin D₂ daily from early in the second week of
gestation, the chorioallantoic placentae showed a delay in trophoblastic matur-
ation and blood vessel formation in the villi. Calcification was detected around
fetal and maternal blood vessels.
Mutagenicity

The Select Committee is not aware of any experimental data concerning the mutagenic activity of vitamin D that have significant bearing on the health aspects of vitamin D as a food ingredient.

Carcinogenicity

Barry et al. (88) applied 0.3 percent ergosterol and calciferol in benzene twice weekly to the skin of mice and found no tumors (epitheliomas, papillomas) after 600 days. Jones et al. (89) considered whether vitamin D-induced hypercalcemia might affect the incidence of tumor metastases in rats. Normal and thyroparathyroidectomized Sprague-Dawley rats were given subcutaneous injections of 20,000 IU of water-soluble vitamin D₃ (about 130,000 IU per kg per day) on alternate days for one week. The animals were then given injections of Walker sarcoma cells. Seventeen days following the tumor inoculations the animals were sacrificed and examined for the presence of gross tumors in the liver and mesentery. Hypercalcemia occurred in all animals but there was no change in the incidence of tumor takes in the animals given vitamin D₃ as compared to controls. Questions concerning the possible carcinogenicity of large amounts of vitamin D were reviewed in 1950 by Touraine and Zureick (90) who concluded that the evidence was negative. However, they suggested that further study in animals and accumulation of data from clinical experiences and observations were warranted.

Interaction with drugs and vitamin A

The biologic action of vitamin D₂ and vitamin D₃ requires several metabolic steps, some of which include protein synthesis (27). Thus, drugs and related substances which affect any of the metabolic steps also affect the manifestation of activity from ingested vitamin D. For example, actinomycin D and cycloheximide, well known for their effects in certain metabolic pathways, inhibit the functioning of 1,25-(OH)₂ vitamin D₃.

Several authors have reported an increased incidence of rickets in children regularly treated with multiple doses of anticonvulsant drugs; in the adult the interaction may lead to hypocalcemia or osteomalacia (27, 91-94). The abnormalities reported are very similar to those observed in vitamin D deficiency (95-98). Hypophosphatemia, lowered concentrations of circulating vitamin D metabolites, increase in alkaline phosphatase, lowered intestinal absorption of calcium, and lowered bone mass have also been reported (99-102). Richens and Rowe (91) reported pheneturide, primidone, phenytoin and phenobarbital as the anticonvulsant drugs most commonly associated with hypocalcemia. Vitamin D supplementation was effective in the treatment of both rachitic and osteomalacic changes in bone (27). These observations led to the study of the effects of phenytoin (Dilantin®) on the metabolism of the labeled vitamin D₃ and 25-(OH)-vitamin D₃. Short-term experiments in rats showed
that both vitamin D₃ and its metabolite disappeared more rapidly from the serum of phenytoin-treated animals than of controls. Villarealle et al. (103) studied interactions between vitamin D₃ and phenytoin in chicks and concluded that phenytoin acts on the metabolism of vitamin D₃ or on tissue responses to the vitamin. Gascon-Barré and Côté (104) have shown that a 21-day pretreatment of rats with phenobarbital or phenobarbital-phenytoin, but not phenytoin alone, increased the LD₅₀ of acutely administered vitamin D₃; the median time to effect was also prolonged. Both of these findings are consistent with an effect of these anticonvulsants on vitamin D₃ metabolism. Gascon-Barré and Glorieux (105) have shown that the total excretion of tritiated 25-(OH)-vitamin D₃ is greater in phenobarbital-treated animals than in controls.

The interrelationships between combined hypervitaminosis A and D were explored by Clark and Bassett (106). Young and adult rats were intubated with vitamin D₃ and vitamin A palmitate in sesame oil. In animals dosed daily with 18,000 IU vitamin D₃ (about 180,000 IU per kg) and 30,000 IU vitamin A for 60 days the growth rate and survival rate were better than in other rats receiving the same amount of vitamin D and 3,000 IU of vitamin A or less, and there was less calcification in the soft tissues and less osteolytic action in the animals given the higher dose of vitamin A. There were no animals given vitamin A without large amounts of vitamin D. In a study by Taylor et al. (107) day-old chicks were fed for 4 weeks on diets containing 4 levels of vitamin D and vitamin A (1, 10, 100, and 1000 times the basal level in all 16 combinations), with the object of investigating a possible antagonism between the two vitamins. At level 1 the chicks were consuming, at the start of the experiment, about 750 IU of vitamin D per kg body weight per day and at level 1000, about 750,000 IU per kg per day. Only diets containing 1000 times the basal level of one or both vitamins depressed growth and induced significant changes in blood calcium, inorganic phosphate and acid phosphatase.

Views of official bodies

In 1963 the Committee on Nutrition of the American Academy of Pediatrics (9) reviewed the relevant literature on vitamin D and issued a statement of policy. In part, the Committee concluded that because of the prevalent practice of food fortification in the United States and Canada, there was a definite possibility that the individual, even the young infant, may ingest considerably more than the recommended vitamin D allowance, and intakes of 2,000 or 3,500 IU per day were possible, particularly beyond infancy. The Committee also pointed out that although there has been no specific evidence that intakes of this order produce deleterious effects beyond infancy, the long-term consequences of this new nutritional situation on older children or adults are entirely unknown. The Committee stated that the practice of enriching foods other than milk and infant formula products is not justified, and discontinuation of fortifying other foods was recommended.
In 1967 a review and policy statement on vitamin D were prepared by Fraser in consultation with the Committee on Nutrition of the American Academy of Pediatrics and endorsed by it (66). It was concluded that the evidence concerning infantile hypercalcemia did not alone provide justification for extensive change in national policies relating to vitamin D. However, it was recommended that there be sensible moderation in consumption of vitamin D at all ages because the potential toxicity of vitamin D after long-term intakes, that exceed requirements by several orders of magnitude, was still unknown.

In 1973 the Food and Nutrition Board of the National Academy of Sciences (108) issued a policy statement, superceding theirs of 1968, on improvement of the quality of foods. The Board continued its endorsement of the enrichment, fortification, and restoration of the nutritional value of certain foods, including the addition of vitamin D to milk, fluid skim milk and nonfat dry milk.

In 1975 the Committee on Nutritional Misinformation of the Food and Nutrition Board (109) issued a report which included the conclusion that "excessive amounts" of vitamin D are hazardous and only individuals with diseases affecting vitamin D absorption or metabolism require more than 400 IU per day; such needs should be established by clinical evaluation, and treatment should be specifically recommended and supervised by physicians.

It is to be noted that in 1977 the Food and Drug Administration (110) proposed deletion of the provision for use of vitamin D as an optional ingredient in enriched rice, pointing out that such addition would serve only to increase the excessive levels of intake of the vitamin.
V. OPINION

In the absence of adequate exposure to sunlight or equivalent light, dietary intake of vitamin D is required for maintenance of health. Vitamin D occurs naturally in fish and fish oils, eggs, liver and dairy products. The amounts naturally present in dairy products are generally inadequate to meet the requirement and vitamin D₃ is added to evaporated milk, infant formulas, and to most fresh fluid cow milk sold by dairies. In addition, vitamin D₂ or vitamin D₃ is commonly added to margarines, to certain breakfast cereals and to a few other foods. Fortification of milk with vitamin D₃ since the 1920s has been credited with the marked reduction in incidence of rickets.

The estimated requirement for vitamin D in the absence of exposure to ultraviolet light is believed to be 100 to 200 IU per day and the Recommended Dietary Allowance of the Food and Nutrition Board, National Research Council, is 400 IU per day. From food sources of vitamin D (naturally occurring or added) it is unlikely that an infant would receive more than 1000 IU per day (perhaps 200 IU per kg per day), a preschool child more than 2000 IU per day (less than 200 IU per kg per day) or an adult more than 5000 IU per day (less than 100 IU per kg per day). However, better estimates of current intakes of vitamin D from dietary sources should, in due course, be developed.

Unequivocal manifestations of vitamin D toxicity including vascular effects have not been reported from consumption of foods including foods fortified with the vitamin. Observations on patients undergoing vitamin D therapy have shown that intakes of vitamin D 1000 IU per kg per day or more (at least 60,000 IU per day for a 60 kg adult) have in some instances been associated with evidence of toxicity. Studies of individuals with disorders qualifying them for disability pensions suggest that long-term ingestion of vitamin D in excess of 1000 IU per day may be a factor in the occurrence of myocardial infarction. However, for the adult there is relatively little likelihood of consumption of such amounts of vitamin D from that currently added to food.

The only suggestions that toxic effects may be produced by intakes of vitamin D less than 1000 IU per kg per day concern two special problems of infants: (1) such intakes may interfere with linear growth, and (2) the rare disorder, idiopathic hypercalcemia, may be caused by or aggravated by such intakes. For reasons detailed in the body of this report, the Select Committee finds unconvincing the few reports which attribute interference in linear growth to intakes of vitamin D less than 1000 IU per kg per day. The incidence of idiopathic hypercalcemia is estimated to be 1 in 20,000 births for all forms of the disorder and 1 in 275,000 births for the severe form. This incidence is low and must be considered in relation to the demonstrated desirability of fortifying foods with vitamin D. The Committee recognizes also that vitamin D intake may aggravate the manifestations of idiopathic hypercalcemia but considers it unlikely that there is a causal relationship.
At the same time, it is evident that the margin of safety between intakes currently achieved by some infants from all sources (perhaps 200 IU per kg per day) and the amounts (1000 to 3000 IU per kg per day) that may produce toxic manifestations in otherwise normal infants is relatively low. There is strikingly little information on the effects of moderate overdoses of vitamin D, particularly in the range of about 200 to 3000 IU per kg per day for all age groups. Additional data are needed for evaluation of the safety of vitamin D in this dosage range. Moreover, evaluations should recognize that relatively long periods are required for adverse effects of vitamin D to become recognizable. Thus, there appears to be need to limit intakes of vitamin D from all sources, including food and vitamin preparations.

In light of these considerations the Select Committee concludes that:

There is no evidence in the available information on vitamin D₂ and vitamin D₃ that demonstrates or suggests reasonable grounds to suspect a hazard to the public when they are used in food 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.
VI. REFERENCES CITED

1. Tracor Jitco, Inc. 1974. Monograph on vitamin D. Submitted to Food and Drug Administration under DHEW contract no. FDA 72-100. [393 pp.].


-31-


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 decisions 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., Senior 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

Reported submitted by:

DECEMBER 20, 1978
Date

George W. Irving, Jr., Chairman
Select Committee on GRAS Substances
PUBLIC HEARING ON VITAMIN D₂ AND VITAMIN D₃

HELD SEPTEMBER 25, 1978*

One request for a hearing was received from Vitamins, Inc., 200 East Randolph Drive, Chicago, Illinois. Subsequent to public announcement of the hearing in the Federal Register, this request was withdrawn and written material was submitted by Vitamins, Inc., in lieu of appearance to make an oral presentation. The hearing was convened, and the public was invited to make oral presentations of data, information, and views concerning the safety of vitamin D₂ and vitamin D₃ as food ingredients that would supplement that available to the Select Committee as reflected in its tentative report. None requested opportunity to be heard.
