EVALUATION OF THE HEALTH ASPECTS OF PYRIDOXINE AND
PYRIDOXINE HYDROCHLORIDE AS FOOD INGREDIENTS

1977

Prepared for

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

Contract No. FDA 223-75-2004
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Life Sciences Research Office
Federation of American Societies
for Experimental Biology
9650 Rockville Pike
Bethesda, Maryland 20014
NOTICE

This report is one of a series 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 non-governmental. 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

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I. INTRODUCTION

This report concerns the health aspects of using pyridoxine and pyridoxine hydrochloride 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 1972.* 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 July 26, 1977 (42 FR 38017 and 38018) 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 pyridoxine and pyridoxine hydrochloride as food ingredients. The Select Committee received no requests for such a hearing on pyridoxine and pyridoxine hydrochloride.

As indicated in the Food, Drug, and Cosmetic Act [21 USC 321(s)], GRAS substances are exempt from the premarking 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 that it is impossible to provide assurance that any substance is absolutely safe for human consumption.

*The document (PB-241 960/4) 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 Select 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 Select 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 Select Committee, there are insufficient data upon which to base a conclusion. The Select 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 pyridoxine and pyridoxine hydrochloride 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

Pyridoxine, 3-hydroxy-4,5-dihydroxymethyl-2 methylpyridine, is a colorless, crystalline solid with a melting point of 160°C. It is soluble in water, alcohol, and acetone but only slightly soluble in ether and chloroform. Its hydrochloride is a water soluble, ether and chloroform insoluble, white, crystalline solid that melts at 206-208°C with decomposition (3).

Pyridoxine and two derivatives, pyridoxal and pyridoxamine, are collectively called vitamin B₆ which is a dietary essential (4).
In adults, dietary deprivation of vitamin B₆ may result in depression and confusion, electroencephalographic abnormalities and convulsions (5). Vitamin B₆-responsive anemia has been reported in adults as well as in infants (6-8). Dietary deprivation of vitamin B₆ in infants may result in epileptiform convulsions, microcytic hypochromic anemia, loss of weight and abdominal distress, vomiting, and hyperirritability (5). Inborn errors of metabolism responsive to administration of vitamin B₆ are well documented and are discussed later in this report (9).

Commercially, vitamin B₆ is added to foods in the form of synthetic pyridoxine hydrochloride. It is stable to heat in acid and alkaline solutions but is light sensitive in neutral or alkaline solution (10). The Food Chemicals Codex (11) specifies that pyridoxine hydrochloride assay not less than 98.0 percent C₆H₁₁NO₃·HCl and contain not less than 16.9 percent or more than 17.6 percent Cl. Heavy metals (as Pb) are limited to 30 ppm. Dietary sources of vitamin B₆ include eggs, yeast, liver, kidney, muscle, fish, milk and milk products, green vegetables, whole grains, and legumes. Schroeder (12) has reported concentrations of vitamin B₆ to average 4.2 µg per g in meats and poultry, 3.5 in fish and seafood, 1.7 in root vegetables, 2.4 in legumes, and 1.7 in green vegetables. In comparing raw and canned products, canning resulted in vitamin B₆ loss of 43 percent in meats, 49 percent in seafoods, 63 percent in root vegetables, 77 percent in legumes, and 57 percent in green vegetables. Of 83 commercially prepared strained or chopped baby foods, 29 percent had a vitamin B₆ content of 2 µg per g or higher.

In most foods, vitamin B₆ occurs principally bound to proteins. However, of the vitamin B₆ in whole milk, 14 percent is in the bound form and 86 percent in the free form. The greater part (70 to 95 percent) of the vitamin is present as pyridoxal, with the remainder in the form of pyridoxamine. The concentration of vitamin B₆ in whole milk averages about 540 µg per liter. Significant decrease in content of vitamin B₆ does not occur during pasteurization or homogenization of milk or in the manufacture and storage of dried whole milk, dried skim milk, or instant non-fat dried milk. However, large losses may occur during heat sterilization of milk (13).

The active coenzyme form of vitamin B₆, pyridoxal-5-phosphate, is required for function of more than 60 enzymes including amino acid decarboxylases, transaminases, racemases, and enzymes involved in tryptophan and cysteine metabolism (14). Enzymes participating in the metabolism of sulfur amino acids appear to be most affected by vitamin B₆ deficiency (15). Kynureninase, homoserine dehydratase (cystathionase) L-serine dehydratase (cystathionine synthetase), threonine dehydratase, L-glutamic acid decarboxylase and 5-hydroxytryptophan decarboxylase also
appear to be affected in the relatively early stages of vitamin $B_6$ deficiency. The various transaminases seem less sensitive to vitamin $B_6$ deficiency.

Pyridoxine, pyridoxal, and pyridoxamine are present in tissues as phosphate esters (16). Pyridoxal also appears to be present in plasma (17, 18).

A number of compounds with chemical similarity to pyridoxine are antagonists of pyridoxine: 4-deoxypyridoxine, 5-deoxypyridoxine, 5-deoxypyridoxal and 5-deoxypyridoxamine (19). In addition, isonicotinic acid hydrazide (isoniazid), an agent effective in treatment of tuberculosis, combines with pyridoxal or pyridoxal phosphate to form a hydrazone that may be lost in the urine or prevent production of pyridoxal phosphate (20). The effectiveness of pyridoxine in counteracting the adverse effects of isoniazid has been well established in mice (21) and humans (22).

The Food and Nutrition Board (5) has set the recommended dietary allowance (RDA) for vitamin $B_6$ at 2 mg per day for adults and 2.5 mg per day during pregnancy. However, it is suggested that considerably larger amounts are necessary in women receiving steroid contraceptives to prevent or correct such changes as increased urinary excretion of tryptophan metabolites. The Board has concluded that satisfactory data are not available to permit establishing daily vitamin $B_6$ requirements for infants and adolescents. The allowances recommended range up to 2.0 mg daily.

Pyridoxine hydrochloride appears among substances that are generally recognized as safe in the Code of Federal Regulations (2) as a nutrient and/or dietary supplement [21 CFR 182.5676]. Pyridoxine base (pyridoxine) is GRAS by prior sanction (23).

III. CONSUMER EXPOSURE DATA

A subcommittee of the National Research Council (NRC) surveyed manufacturers in 1970 concerning the level of addition of pyridoxine hydrochloride to foods (24). Based on information supplied, a weighted mean was calculated (Table I) for the usual addition level of pyridoxine hydrochloride to food products in several categories.

The NRC subcommittee estimated possible average daily intakes (Table II) from Market Research Corporation of America data on mean frequency of eating foods by food category, U.S. Department of Agriculture data on mean portion size of foods in these categories and the assumption that all foods within a category contain the substance at the level shown in Table I. Such an assumption is likely to lead to overestimates of intake.
### TABLE I

**Level of Addition of Pyridoxine Hydrochloride to Foods by Food Category (24)**

<table>
<thead>
<tr>
<th>Food category</th>
<th>Weighted mean ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baked goods, baking mixes</td>
<td>10</td>
</tr>
<tr>
<td>Breakfast cereals</td>
<td>29</td>
</tr>
<tr>
<td>Milk, milk products</td>
<td>28</td>
</tr>
<tr>
<td>Meat products</td>
<td>8</td>
</tr>
<tr>
<td>Snack foods</td>
<td>3</td>
</tr>
<tr>
<td>Reconstituted vegetable proteins</td>
<td>4</td>
</tr>
<tr>
<td>Dairy products analogs</td>
<td>20</td>
</tr>
<tr>
<td>Baby baked goods</td>
<td>50</td>
</tr>
<tr>
<td>Baby cereals</td>
<td>2</td>
</tr>
<tr>
<td>Baby formulas</td>
<td>1</td>
</tr>
</tbody>
</table>

Level of addition of pyridoxine hydrochloride 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 24.

### TABLE II

**Possible Average Daily Intake of Added Pyridoxine Hydrochloride by Age Group (24)**

<table>
<thead>
<tr>
<th>Age group</th>
<th>mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-5 mo</td>
<td>0.5</td>
</tr>
<tr>
<td>6-11 mo</td>
<td>2.9</td>
</tr>
<tr>
<td>12-23 mo</td>
<td>3.1</td>
</tr>
<tr>
<td>2-65+ yr</td>
<td>3.7</td>
</tr>
</tbody>
</table>
The NRC subcommittee has recognized that in most cases its calculations of possible intakes are overstated, often by considerable margins. Because of factors detailed in Section XI of the subcommittee's report (24), it stated that the average estimated dietary intakes are likely to be much higher than intakes that would be achieved through consumption of a diet consisting entirely of processed foods to which the substance had been added at maximum levels.

The estimates in Table II should be viewed in the light of estimates made by the same NRC subcommittee concerning total amounts of pyridoxine hydrochloride reported by the manufacturers to be used in foods. As may be seen from Table III, the per capita "intake" from this source in 1970 was approximately 0.2 mg per day. The Select Committee believes that the estimated intake of 3.7 mg per day of pyridoxine hydrochloride for individuals over two years old (Table II) is excessive and that actual intakes resulting from the amounts added to foods are more likely to be in the range of 0.2 mg per day, as indicated in Table III. This belief is supported by estimates of the total daily dietary intake of vitamin B₆ (25). The daily intake in 1965 and 1966 of adult males, depending on age, was 1.3 to 2.1 mg; adult females 1.1 to 1.3 mg.

The intakes of infants and children less than two years old, indicated in Table II, are also likely to be substantially overestimated. For infants less than six months of age, the major intake of pyridoxine added to foods is likely to be from commercially prepared infant formulas. Of the formulas most widely utilized most provide 0.4 mg of pyridoxine hydrochloride per liter at standard dilution (26, 27). Some infant formulas provide as much as 0.6 mg of added pyridoxine hydrochloride per liter at standard dilution. The small percentage of infants who might consume one liter daily of a formula providing 0.6 mg of added pyridoxine hydrochloride, would therefore consume 0.6 mg of pyridoxine hydrochloride from this source (about 0.1 mg per kg per day). The great majority of infants less than five months of age consume less than 0.4 mg per day of pyridoxine hydrochloride added to foods.

*An explanation for such overstatements is detailed in Section XI, "Significance and Use of Data in Safety Evaluations," of the NRC subcommittee's report (24). The Select Committee finds this explanation reasonable and concurs in the first recommendation in Section XII of the same report, that "In order to conduct a more accurate survey of the intake of substances used in food processing, food consumption data collected specifically for this purpose are needed."
TABLE III

Quantity of Pyridoxine Hydrochloride Added Annually to Foods and Per Capita Daily "Intake" Calculated Therefrom

<table>
<thead>
<tr>
<th>Relative quantities used¹</th>
<th>Total quantity used (1970)²</th>
<th>Per capita daily &quot;intake&quot;³</th>
</tr>
</thead>
<tbody>
<tr>
<td>1970/1960</td>
<td>kg</td>
<td>mg</td>
</tr>
<tr>
<td>7</td>
<td>13,100</td>
<td>0.2</td>
</tr>
</tbody>
</table>

¹ Based only on the reports from those respondents to the National Research Council (NRC) survey who submitted information for both 1960 and 1970 (24).

² Total usage is based on the sum of the amounts used in foods as supplied by NRC and the Flavor and Extract Manufacturers' Association (FEMA) recalculated to 100 percent from survey data that the NRC subcommittee estimated to represent about 60 percent of the actual usage.

³ Based on a U.S. population of 205 million.

IV. BIOLOGICAL STUDIES

Absorption, metabolism, distribution, and excretion

Vitamin B₃ compounds are readily absorbed; after oral administration of a 50 or 100 mg dose of pyridoxine hydrochloride (about 0.8 to 1.6 mg per kg body weight), maximum concentration of pyridoxal-5-phosphate in the blood is reached in two hours in men and in pregnant and nonpregnant women and an elevated blood level continues for at least four days. About 30 percent of the administered dose of pyridoxine hydrochloride is excreted as the metabolites pyridoxal and 4-pyridoxic acid or as pyridoxine hydrochloride in the urine within 24 hours after administration (28, 29). Rapid absorption and metabolism of pyridoxine hydrochloride are also apparent from the prompt increase in urinary excretion of metabolites after oral administration of smaller doses to man and animals (30-32).

Studies with oral doses of 0.05 to 5.0 mg of tritiated pyridoxine hydrochloride in rats indicate that absorption, presumably by diffusion, occurs primarily in the jejunum, although some absorption occurs in the ileum and a small amount in the colon (33). Based on studies of patients with intestinal resections, it is suggested that in the human, absorption of pyridoxine occurs primarily in the jejunum (34).
The major metabolite of vitamin B₆ excreted in the urine by human subjects is 4-pyridoxic acid (29, 30, 35). Contractor and Shane (35) have suggested that 4-pyridoxic acid may be formed by either the generally accepted pathway from pyridoxal phosphate to pyridoxal and then, through the action of an aldehyde oxidase to 4-pyridoxic acid or, by the pathway that the authors believe may be predominant, from pyridoxal phosphate via 4-pyridoxic acid-5-phosphate to 4-pyridoxic acid. According to Contractor and Shane, failure of earlier investigators to recognize the presence of the metabolite, 4-pyridoxic acid-5-phosphate in the urine may help to explain some of their relatively low urinary recoveries of administered doses of vitamin B₆.

Dahlkvist et al. (36) administered [³H₆]pyridoxine intravenously to NMRI mice and analyzed the tissues at intervals thereafter. Initial concentrations of the isotope were high in all tissues except brain. One day after injection, isotope concentration was greatest in liver, heart, and kidney; seven days after injection, concentration was greatest in liver but substantial concentrations were also found in heart, carcass, muscle, brain, and kidney, with lesser concentrations in lung, intestine, and spleen. Between 5 and 30 days after injection about 75 percent of the total tissue activity was located in carcass and 10 to 15 percent in liver. From the slopes of the curves describing total isotope content of the body at various times after injection, the rate of elimination of the vitamin was found to be 7.9 percent per day (5.3 percent per day for carcass and 8.7 percent per day for liver); the biologic half-life of vitamin B₆ in the body was estimated to be about 10 days. The rate of elimination of the vitamin determined in this way was greater than that (6.5 percent per day) calculated on the basis of rate of urinary excretion. On this basis, it seems likely that an earlier estimate of a half-life in man of 18 to 38 days for pyridoxine (equivalent to a daily loss of 2 to 3 percent per day) may be somewhat excessive unless there are substantial species differences (30). Such differences are known to exist for some other nutrients.

In animal tissues, vitamin B₆ has been reported to occur mainly in the form of pyridoxal-5-phosphate and pyridoxamine-5-phosphate (37). After intravenous administration of [³H₆]pyridoxine (0.8 mg per kg body weight) to mice, pyridoxine disappeared from the liver within 60 minutes. The concentration of pyridoxine-5-phosphate reached a maximum in the liver at 10 to 15 minutes and then decreased. By 60 minutes after injection, less than 20 percent of radioactivity was accounted for by pyridoxamine-5-phosphate and more than 60 percent was accounted for by pyridoxal-5-phosphate. Small amounts of pyridoxal and pyridoxamine were also present. Similar changes in ratios of pyridoxamine-5-phosphate to pyridoxal-5-phosphate occurred in carcass but the transformations occurred at a slower rate. Between one and seven days after injection the ratio of pyridoxal and pyridoxamine phosphates remained constant, pyridoxal-5-phosphate accounting for about 50 to 60 percent of the radioactivity in liver and pyridoxamine-5-phosphate.
accounting for about 30 to 40 percent (36). It should be noted that in these studies, the existence of 4-pyridoxic acid-5-phosphate, later identified by Contractor and Shane (35), was not considered.

Utilizing $[^{14}\text{C}]$ pyridoxine, McCoy and Colombini (38) studied interconversions of vitamin $B_6$ in liver, brain, and carcass of male Swiss-Webster mice injected intravenously with 115 $\mu$g of vitamin $B_6$. They concluded that the principal reactions in liver were conversion of pyridoxine to pyridoxal-5-phosphate and pyridoxamine-5-phosphate and a continued interconversion between these two compounds. The total radioactivity retained in liver, 24 hours after injection was almost equally divided between pyridoxal-5-phosphate and pyridoxamine-5-phosphate. Whereas the initial conversion in liver was from pyridoxine to pyridoxamine-5-phosphate, in brain the initial conversion was about equal to pyridoxamine-5-phosphate and to pyridoxal-5-phosphate. A smaller percentage of total radioactivity was retained in brain than in liver 24 hours after injection, and was subsequently lost more slowly than from liver. In carcass, the initial conversion appeared to be oxidation to pyridoxal. Twenty-four hours after injection, 7 percent of the injected dose was present in carcass compared with 1.6 percent in liver and 0.1 percent in brain. These data were considered to support the hypothesis that carcass is a storage site for vitamin $B_6$ in the body.

Tiselius (39) has demonstrated that the sequence of metabolic transformation of intravenously injected tritium-labeled pyridoxine in rat brain was pyridoxine to pyridoxine-5-phosphate to pyridoxal-5-phosphate to pyridoxamine-5-phosphate. After the initial transformation period about 40 percent of the isotope was recovered as pyridoxal-5-phosphate and about 40 percent as pyridoxamine-5-phosphate.

Administration of pyridoxine hydrochloride to patients receiving levo-dihydroxyphenylalanine (L-DOPA) for Parkinson's disease decreased the beneficial central system effects of L-DOPA (40-43) but at least in the one case studied, alleviated torsion dystonia, one of the side-effects of L-DOPA therapy (44). The mechanism of this effect is believed by some to be an increase in decarboxylation of L-DOPA to dopamine peripherally, with a consequent decreased availability of L-DOPA to the brain (41,45). However, it is perhaps more likely that the antagonism reflects the formation of a Schiff base between pyridoxine and L-DOPA, resulting in a general biochemical and pharmacological inactivation of L-DOPA and pyridoxal phosphate (41,46).

In vivo incorporation of $[^{14}\text{C}]$ acetate into liver cholesterol is increased in the vitamin $B_6$-deficient rat (47,48). Administration of vitamin $B_6$ subcutaneously, 6 mg per kg per day, to normal rabbits did not result in any sustained significant changes in $[^{14}\text{C}]$ acetate incorporation into serum or liver cholesterol (49). However, when the diet contained 1 percent cholesterol, incorporation of $[^{14}\text{C}]$ acetate into cholesterol in serum and liver
was decreased. In the human, a slight but statistically significant decrease in serum cholesterol was reported in diabetic and non-diabetic subjects given oral doses of 400 mg of pyridoxine daily for 5 to 36 days (50). Goldner and Vallan (51) reported that administration of pyridoxine significantly potentiated the serum cholesterol-lowering effect of niacin in 53 patients.

**Acute toxicity**

**Animals.** A summary of data on acute toxicity of pyridoxine hydrochloride and pyridoxine base in the mouse and the rat is presented in Table IV. The LD₅₀ for single oral doses of pyridoxine hydrochloride or pyridoxine base ranged from 3000 to 7000 mg per kg for rats (54). Animals receiving sub-lethal doses of pyridoxine or its hydrochloride demonstrated awkward gait, impaired reflexes and convulsions for periods from several days to as long as three weeks. With lethal doses, tonic convulsions occurred with death in 36 to 72 hours.

Based on a study of four dogs, the lethal oral dose appears to be 1000 to 2500 mg per kg (55). Oral administration of 3000 to 7000 mg per kg of pyridoxine to rats or 1000 to 2500 mg per kg of pyridoxine hydrochloride to dogs resulted in histologic abnormalities of the nervous system (mainly rarefaction of the posterior columns) and death (55). Dogs given 1000 mg per kg developed ataxia but recovered within a week (53).

The ratio between the requirement of rats for pyridoxine (approximately 10 μg per day for animals weighing 200 g) and the LD₅₀ (approximately 1 g per animal) is 1 to 100,000 (56). This ratio of requirement to the oral LD₅₀ for pyridoxine compares to 1:30,000 for thiamin, 1:≥2000 for riboflavin, 1:≥8000 for nicotinic acid, and 1:≥20,000 for pantothenic acid.

Rabbits and cats anesthetized with urethane and chloralose demonstrated no change in respiration, heart rate or blood pressure after intravenous injection of vitamin B₆ in dosage of 1 to 50 mg per kg (53).

**Humans.** No adverse manifestations were noted after oral or parenteral administration of 100 to 200 mg of pyridoxine hydrochloride (about 1.7 to 3.5 mg per kg body weight) to young male adults (52). Five subjects received 100 mg orally, six received 100 mg intramuscularly, six received 100 mg intravenously, and three received 200 mg intravenously.

**Short-term studies**

**Animals.** No pathological changes were detected in five mice given intravenous injections of pyridoxine hydrochloride, 100 mg per kg, daily except weekends for two weeks (52). No toxic effects were observed in 20 young rats fed 25 mg of pyridoxine (probably >500 mg per kg) daily for 100 days (53).
TABLE IV

Acute Toxicity in Mouse and Rat (LD$_{50}$)

<table>
<thead>
<tr>
<th>Substance</th>
<th>Species</th>
<th>Number of animals at each dose level$^1$</th>
<th>Route</th>
<th>Dosage, mg/kg body weight</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyridoxine hydrochloride</td>
<td>Mouse</td>
<td>23$^2$</td>
<td>i.v.</td>
<td>545</td>
<td>52</td>
</tr>
<tr>
<td>Pyridoxine hydrochloride</td>
<td>Rat</td>
<td>23$^2$</td>
<td>i.v.</td>
<td>658</td>
<td>52</td>
</tr>
<tr>
<td>Pyridoxine</td>
<td>Rat</td>
<td>10</td>
<td>s.c.</td>
<td>3100</td>
<td>53,54</td>
</tr>
<tr>
<td>Pyridoxine hydrochloride</td>
<td>Rat</td>
<td>10</td>
<td>s.c.</td>
<td>3700</td>
<td>53,54</td>
</tr>
<tr>
<td>Pyridoxine</td>
<td>Rat</td>
<td>10</td>
<td>oral</td>
<td>4000</td>
<td>54</td>
</tr>
<tr>
<td>Pyridoxine hydrochloride</td>
<td>Rat</td>
<td>10</td>
<td>oral</td>
<td>5500</td>
<td>53,54</td>
</tr>
<tr>
<td>Pyridoxine hydrochloride</td>
<td>Rat</td>
<td>10</td>
<td>oral</td>
<td>6000</td>
<td>54</td>
</tr>
</tbody>
</table>

$^1$ Sex not indicated

$^2$ Total number of animals used
Fourteen young male rats received daily intramuscular injections of 50 mg of pyridoxine (approximately 700 mg per kg) and two rats were sacrificed every four days for 28 days (57). Slight initial hypertrophy of the adrenals was observed with persistent adrenal histologic abnormalities and some atrophy of the thymus. Early in the study, moderate granulocytosis was observed with relative lymphopenia. However, results were not tabulated as to changes in organ weights or the induction of the profound hematopoietic shift from the normal predominance of agranulocytes (70 to 75 percent) in the circulating blood of the rat to the "moderate granulocytosis with relative lymphopenia" reported; histochemical evaluation of the adrenal cortical changes was not performed. Doses of pyridoxine were very high and were administered intramuscularly rather than orally.

Twenty-six male Nelson-Wistar rats, weighing 50 to 60 g at the onset of the experiment, were fed a diet providing a large amount of pyridoxine hydrochloride, about 50 mg per kg body weight per day, for five weeks (58). An equal number of pair-fed controls consumed about 5 mg per kg body weight per day of pyridoxine hydrochloride. Gain in weight was approximately 20 percent greater and liver weight approximately 40 percent greater for the animals receiving the higher intake of the vitamin. Relative weights of brain, pancreas, and kidney were unaffected. Rats consuming the diet, which was exceptionally high in pyridoxine hydrochloride, differed from controls in that incorporation of $^{35}$S from cystine into reduced glutathione in liver was increased whereas incorporation into oxidized glutathione was decreased. No significant differences between the high pyridoxine and control animals were found in concentrations of amino acids or activities of cystathionase and cystathionase in liver, kidney, pancreas, and brain. Because the 5 mg per kg of body weight per day of pyridoxine hydrochloride consumed by the controls represented a relatively high intake, six rats were pair-fed a diet providing approximately 0.25 mg per kg of body weight per day of pyridoxine hydrochloride. No significant differences in gain in weight, gain per unit of food intake or liver weight were found between the two sets of control animals.

Humans. Six inherited disorders known to demonstrate vitamin B$_6$ dependency have been identified: infantile convulsions, pyridoxine-responsive anemia, cystathioninuria, xanthurenicaciduria, homocystinuria and hyperoxaluria (9). All of these require 5 to 50 times the usual physiologic dose of vitamin B$_6$ for biochemical and/or clinical improvement although there is scant evidence at present that this therapy is worthwhile (59). Long-term administration of large doses of pyridoxine hydrochloride to patients with these disorders appears not to be associated with adverse reactions. Doses of 100 to 1500 mg of pyridoxine hydrochloride daily have been administered for periods of a few months to three or four years to patients with homocystinuria (60-62) and other vitamin B$_6$ dependency syndromes (6-8, 63).
Long-term studies

A study of three-generations of rats was carried out by Unna (53), with 13 rats in the first generation, 13 in the second generation, and 19 in the third generation. These rats received 2.5 mg of pyridoxine hydrochloride daily by mouth from the time of weaning at 21 days of age. This dose amounted to approximately 90 mg per kg body weight at weaning and 10 mg per kg for males and 14 mg per kg for females after 80 days of study. Weight gain was the same as that of 14 control animals receiving stock diet and the young grew normally in each generation. Two litters were obtained from the first generation (which included four females) and three litters were obtained from the second generation (which included six females). No observations were recorded concerning reproductive performance.

Reproduction

In 1954 Hunt et al. (64) had speculated that pyridoxine-dependency in the newborn might result from administration of large doses of pyridoxine hydrochloride to the pregnant woman. This speculation was based on observations of the child of a woman treated with large (50 mg three or four times weekly) doses of pyridoxine hydrochloride intramuscularly because of severe nausea and vomiting during the second through fifth months of her third pregnancy. The infant born at the termination of the pregnancy demonstrated pyridoxine-dependency manifested by convulsions. Convulsions were eliminated by administration of pyridoxine hydrochloride. The woman's first pregnancy, during which vitamin B₆ was not administered, resulted in a normal infant. Her second pregnancy during which she had received pyridoxine hydrochloride intramuscularly, resulted in an infant who developed convulsions and died. Other cases of presumed pyridoxine-dependency in the infants of women receiving large doses of pyridoxine during pregnancy have not been reported and animal studies have failed to support the hypothesis.

Morrison and Sarett (65) studied the effects of relatively large daily intakes of pyridoxine on growth and reproduction of female weanling rats of the McCollum-Wisconsin strain. Groups of 12 rats, 19 to 21 days old and weighing about 50 g, were given various diets for 12 weeks. The control group received a diet presumably adequate in thiamin and pyridoxine (150 µg pyridoxine per 100 g of diet) which supplied approximately 0.15 mg of pyridoxine per kg of body weight per day. Test animals received 50 times the control concentration of pyridoxine (7.5 mg per kg per day), thiamin, or both. No significant effect on weight gain or food consumption was reported. While continuing to receive the same diets, the rats were mated. The high intakes of pyridoxine appeared to exert no adverse effects on reproductive performance and a greater number of young per litter (9.7) were born to the animals receiving high intakes of both pyridoxine and thiamin than to controls (7.5 per litter). The dams were killed 21 days after parturition. Weights of kidneys, liver and adrenals were unaffected by the intake of pyridoxine.
Concentrations of total solids, lipids, riboflavin, pantothenate, thiamin, pyridoxine and vitamin B₁₂ in these organs were unaffected. The young, weaned at 21 days, were studied for five weeks while receiving a pyridoxine-deficient diet. The young of dams which had received the high pyridoxine dose gained significantly more weight than did the controls. The young were then killed and concentration of pyridoxine in the liver was found to be greater for pups born to and nursed by the dams with high intakes of pyridoxine.

In a study by Schumacher et al. (66), 15 female Long-Evans rats were fed a control diet containing 250 μg of pyridoxine per 100 g of diet (intake of pyridoxine approximately 0.17 mg per kg body weight per day) and five female animals of the same strain were fed a diet containing 25 times this amount of pyridoxine (about 4 mg per kg body weight per day). After two weeks, the test and control rats were mated with untreated males and the same test and control diets were continued throughout gestation and lactation. No significant differences were detected between test and control animals with respect to number of young, birth weight, weight when weaned at age 21 days, or in pyridoxine requirement after weaning.

Groups of 12 female Sprague-Dawley rats weighing 250 g were fed a basal diet supplemented with various concentrations of pyridoxine hydrochloride for one week (67). Half of the animals were than mated and continued on the same diet until they were killed on the twenty-first day of gestation. An intake of as much as 32 mg pyridoxine hydrochloride per kg body weight per day had no effect on maternal food intake, weight gain or liver weight, number of resorptions, number of live young, or fetal weight.

In comparison with 15 controls, 15 pubescent female rats given daily intraperitoneal injections of 0.4 mg pyridoxine hydrochloride per kg body weight for three weeks and, subsequently, 0.8 mg per kg per day for an additional 66 days, demonstrated shortened estrus cycle and no diestrous phase in most cases; estrus phase was prolonged (68). There was hypertrophy of ovaries and uterus and many follicles and corpora lutea were observed. Unfortunately, examples of individual responses were given rather than a tabulation of the responses of all rats, treated and controls. Illustrations of morphological effects were limited to an ovary and a uterus from two different treated rats and were of selected areas in each; these cannot be regarded as depictive of all of the structures claimed as reactive by the authors. No illustrations of control rats' organs were included and organ weights of controls were not measured. Injections were administered intraperitoneally without sham treatment of control rats. Oral administration of pyridoxine at the reported doses would not, in all probability, have induced such changes.
Teratogenesis

Karnofsky et al. (69) have reported on the effects of various vitamin B₈ compounds injected into the yolk sac of fertile eggs of white Leghorn chickens (6 to 20 eggs per dose level) at various stages of incubation. The LD₅₀ for embryos (mg per egg) on the fourth day of incubation was 12.0 for pyridoxine, more than 20.0 for pyridoxamine, 5.0 for pyridoxal and 3.0 to 5.0 for pyridoxal phosphate. On the thirteenth day of incubation, the LD₅₀ was more than 20.0 for pyridoxine and pyridoxamine and 5.0 for pyridoxal. Pyridoxal phosphate was not tested. Embryonic abnormalities were not consistently found.

Injections of 0.1 to 1.0 mg of pyridoxine into the albumin of fertilized eggs of Leghorn chickens (103 to 106 eggs at each of five dosage levels) 24 hours after onset of incubation did not result in greater frequency of embryonic deaths, underdeveloped embryos or deformities than was observed in controls (70).

Carcinogenesis

No studies of the carcinogenicity of pyridoxine, pyridoxal or pyridoxamine have come to the attention of the Select Committee. However, it has been reported that implanted Flexner-Jobling carcinoma in Sprague-Dawley rats, Yale carcinoma 1 and a fibrosarcoma in ABC mice, and methylcholanthrene-caused skin tumors in mice, were retarded when diets depleted or low in pyridoxine content were administered (71). Similar results were obtained with dimethylaminoazobenzene-induced liver tumors in Sprague-Dawley rats (72, 73). On the other hand, daily parenteral administration of up to 4 mg pyridoxine hydrochloride (about 132 mg per kg body weight per day) was reported to have no effect on the course of leukemia after an intravenously administered leukemic transplant in AK mice (74).

V. OPINION

Vitamin B₈ compounds, after conversion to the active coenzyme form, pyridoxal phosphate, function in a variety of enzyme reactions that are metabolically essential. The vitamin is naturally present in a wide variety of foods. The Recommended Dietary Allowance is 2 mg per day for adults and 2.5 mg per day during pregnancy, although it has been suggested that an intake as high as 15 to 30 mg per day may be desirable for women during pregnancy or those using oral contraceptives. Thus, the daily per capita "intake" of 0.2 mg of pyridoxine hydrochloride per day (0.003 mg per kg per day for adults) resulting from its addition to foods may amount to no more than 10 percent of the total intake.
Available data are inadequate, because of variations in consumption patterns, for estimating the greatest amounts of pyridoxine hydrochloride added to foods that may be consumed by individuals in various age groups beyond infancy. For infants, such variations are rare because a large percentage of the food intake may be derived from commercially prepared formulas fortified with pyridoxine hydrochloride. It is estimated that the largest consumption by infants of pyridoxine hydrochloride added to foods will be less than 0.1 mg per kg body weight per day.

Oral administration of pyridoxine hydrochloride or pyridoxine base to experimental animals in doses of approximately 10 mg per kg per day to more than 100 mg per kg per day has not been associated with adverse effects, including effects on reproductive performance. Pyridoxine dependency has not been produced in offspring of animals given high doses of pyridoxine hydrochloride during pregnancy. The acute LD₅₀ in animals generally ranges from 1000 to 6000 mg per kg when administered orally. Patients with homocystinuria and other vitamin B₆ dependency states have been given daily doses of 1 to 25 mg per kg per day for months without evidence of adverse reactions.

The Select Committee has no evidence that pyridoxine (i.e., pyridoxine base) is added to foods in the United States. Even if it were, its safety threshold appears to be no different than that of pyridoxine hydrochloride.

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

There is no evidence in the available information on pyridoxine hydrochloride or pyridoxine that demonstrates, or suggests reasonable grounds to suspect, a hazard to the public when they are used at levels that are now current or that might reasonably be expected in the future.
VI. REFERENCES CITED


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

Report submitted by:

November 17, 1977
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