EVALUATION OF THE HEALTH ASPECTS OF TANNIC ACID
AS A FOOD INGREDIENT

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 tannic acid as a food ingredient. 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 1973.* 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, or in lieu of an oral presentation submit a written statement, of data, information and views on the health aspects of using tannic acid as a food ingredient. The Select Committee received no requests for a public hearing but received one statement on tannic acid from General Foods Corporation, Tarrytown, New York.

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 that it is impossible to provide assurance that any substance is absolutely safe for human consumption.

*The document (PB-223 858/2) 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 tannic acid and submits its interpretation and assessment in this report, which is intended for the use of FDA in determining the future status of this substance under the Federal Food, Drug, and Cosmetic Act.

II. BACKGROUND INFORMATION

The Select Committee considers only hydrolyzable gallotannins to be within the definition of tannic acid for the purposes of this evaluation. Condensed tannins are apparently not added to foods as direct food ingredients, and the health aspects of naturally occurring condensed tannins are not evaluated in this report.

Tannins are widely distributed in plants and broadly defined as two groups of polyphenolic substances, hydrolyzable tannins and condensed tannins (3,4). The hydrolyzable tannins are further defined as ellagitannins or gallotannins by the presence or absence, respectively, of ellagic acid in the acid hydrolysate of the tannin. Haslam (3) refers specifically to Chinese gallotannin isolated from Rhus semialata as tannic acid. Singleton and Kratzer (4) refer to tannic acid as a gallotannin that yields glucose and about seven or less gallic acid or m-digallic acid units per glucose molecule upon acid or enzymatic hydrolysis. However, commercial practice applies the term tannic acid generally to hydrolyzable gallotannins including tara tannin which yields quinic acid rather than glucose as a hydrolysis product (5,6). Hydrolyzable gallotannins derived from tara tannin are within the above definition of tannic acid.
Tannic acid is subject to spontaneous hydrolysis as well as hydrolysis by various tannases, hot water, acids and alkali (3). The composition and purity of tannic acid preparations are variable and dependent on the plant source and extraction procedure employed in their preparation. They may contain polymers of gallic acid (trigallic acid), unesterified hydroxyl groups, free gallic acid, and other substances.

Investigators of the toxicity of tannic acid seldom employ identical substances and often fail to identify the substance adequately. These practices make comparing experimental results difficult. When available, the grade and botanical source of the tannic acid used for research are identified in this report. Many studies on tannin extracts; tannins in wine, tea, and coffee; and isolated condensed tannins were considered as not related to an evaluation of food grade tannic acid which should include only hydrolyzable gallotannins. In addition to numerous condensed tannins, many lower molecular weight esters of quinic acid and glucose occur naturally in tea, coffee, wine, grape products, vegetables and fruit (3). Esters of hydroxycinnamic acids are among the more widely occurring of these.

The Food Chemicals Codex (5) gives the following description of food grade tannic acid:

"A tannin usually obtained from nutgalls, the excresences which form on the young twigs of Quercus infectoria Oliver [Turkish or Aleppo tannin (2)], and allied species of Quercus L. (Fam. Fagaceae), or from the seed pods of Tara (Caesalpinia spinosa). It occurs as an amorphous powder, as glistening scales, or as spongy masses, varying in color from yellowish white to light brown. It is odorless or has a faint, characteristic odor and an astringent taste. Tannic acid is very soluble in water, in acetone, and in alcohol, but only slightly soluble in absolute alcohol. It is practically insoluble in benzene, in chloroform, in ether, and in solvent hexane. One gram dissolves in about 1 ml of warm glycerine."

According to the Food Chemicals Codex (5), food grade tannic acid may not contain more than 3 ppm arsenic, 40 ppm heavy metals, or 10 ppm lead. Limits are also specified for gums or dextrin, residue on ignition and resinous substances.

Reagent grade "tannic acid (tannin)" is described, without reference to botanical source, in the United States Pharmacopeia (6). Tannic acid was listed in the National Formulary XII but not admitted to National Formulary XIII; inclusion is based on usefulness in human medicine or as an adjunct relating to human medicine (7).
Tannic acid is on a list of substances presumed to be GRAS by FDA but not published (Appendix A in reference 8). It is also listed as GRAS in the Code of Federal Regulations (2) as a natural extractive from "nut-galls of Quercus infectoria Oliver and related spp. of Quercus. Also in many other plants" [21 CFR 182.20]. Tannic acid is permitted as an agent for refining animal fats under meat inspection regulations (9) but it must be eliminated during the process of manufacturing (9 CFR 318.7). Tannin (including quebracho extract) is regulated as a boiler water additive (2) that may be used in the preparation of steam that will contact food [21 CFR 173.310]. The tannin prepared from the quebracho tree, Schinopsis lorentzii, is a condensed tannin (3); the use of condensed tannins is not evaluated in this report.

Tannic acid has been used by U.S. food processors since 1899 (8). It is used as a clarifying agent in the brewing and wine industries and as a flavoring agent in flavors such as butter, caramel, fruit, brandy, maple, and nut (10). The astringent taste and the property of reacting with and precipitating many proteins are the reasons tannic acid is added to foods. It is purported that most of the tannic acid which is added for refining of food-stuffs is removed as a precipitate (11).

The reaction of tannic acid with proteins involves peptide bonds. Chinese, sumac, Turkish (Aleppo) and tara gallotannins are capable of tanning animal skins (12). The biological consequences of this reaction in foods are not completely described; however, several in vitro studies have been reported. The hydrolysis of casein by trypsin was inhibited when casein was complexed with oak leaf tannins at pH 7.6 (13). Inhibition of hydrolysis was proportional to tannin concentration at pH 9.2, and in this system, condensed tannin was a more effective trypsin inhibitor than hydrolyzable oak leaf tannins. Pan- creatin digestion in vitro of protein in soybean meal at pH 7.6 decreased with increasing levels of Aleppo, sumac, tara, and quebracho tannins (14). In contrast, pepsin digestion of soybean meal at about pH 1 was not affected by the level or type of tannin present. This difference was attributed to an instability of the tannin-protein complex. Van Buren and Robinson (15) have studied tannic acid complexes of gelatin and found the maximum precipitation to occur at pH 4 to 5. Soluble complexes existed at lower pH values.

III. CONSUMER EXPOSURE DATA

In 1970, a subcommittee of the National Research Council (NRC) surveyed manufacturers by questionnaire concerning their usual and maximum levels of addition of tannic acid to foods by food category (8). A subsurvey was conducted by the Flavor and Extract Manufacturers' Association (FEMA) and incorporated into the report of the subcommittee. Weighted means of the usual level of addition of tannic acid in ppm are included in Table I. It is noted that these weighted means do not express the highest amount of
tannic acid added by any manufacturer; they do not indicate that all foods in a category contain added tannic acid, and they do not necessarily coincide with the levels added by any one manufacturer. The tannic acid addition levels and the food categories reported in an earlier FEMA survey (16) are in general agreement with Table I.

TABLE I

<table>
<thead>
<tr>
<th>Food category</th>
<th>Weighted mean</th>
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<tbody>
<tr>
<td>Baked goods, baking mixes</td>
<td>41 ppm</td>
</tr>
<tr>
<td>Frozen dairy desserts, mixes</td>
<td>139 ppm</td>
</tr>
<tr>
<td>Meat products</td>
<td>8 ppm</td>
</tr>
<tr>
<td>Soft candy</td>
<td>8 ppm</td>
</tr>
<tr>
<td>Gelatins, puddings, fillings</td>
<td>20 ppm</td>
</tr>
<tr>
<td>Beverages, nonalcoholic</td>
<td>5 ppm</td>
</tr>
<tr>
<td>Beverages, alcoholic</td>
<td>65 ppm</td>
</tr>
<tr>
<td>Hard candy</td>
<td>131 ppm</td>
</tr>
</tbody>
</table>

The NRC subcommittee estimated possible average daily intakes (Table II) of tannic acid for various age groups from Market Research Corporation of America data on the mean frequency of eating food by food category, U.S. Department of Agriculture data on mean portion size of foods in those categories, and the assumption that all food products within a category contain added tannic acid at the levels shown in Table I (8). Such an assumption is likely to lead to overestimates of intake because most foods do not contain added tannic acid.

The NRC subcommittee has recognized that in most cases its calculations of possible intakes are overstated (8). They stated that the possible average estimated total dietary intakes are likely to be much higher than would be the intakes achieved through consumption of a diet consisting totally of processed foods to which the substances had been added at the maximum levels. A correction for the use of tannic acid as a processing aid in a manner that largely results in its elimination from a product during processing also should substantially lower the estimates of possible daily intakes. However, the Select Committee has no information on the concentration of added tannic acid and its hydrolysis products that remain in foods as consumed.
TABLE II

Possible Average Daily Intake of Added Tannic Acid by Age Groups (8)

<table>
<thead>
<tr>
<th>Age group</th>
<th>mg</th>
<th>mg/kg a</th>
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<tbody>
<tr>
<td>0-5 mo</td>
<td>0.3</td>
<td>0.06</td>
</tr>
<tr>
<td>6-11 mo</td>
<td>2.9</td>
<td>0.36</td>
</tr>
<tr>
<td>12-23 mo</td>
<td>5.1</td>
<td>0.46</td>
</tr>
<tr>
<td>2-65+ yrs</td>
<td>12.9</td>
<td>0.21</td>
</tr>
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</table>

a Calculations based upon average body weight of 60 kg for an adult (17), and the following estimated weights of infants by age groups: 0-5 mo, 5 kg; 6-11 mo, 8 kg; and 12-23 mo, 11 kg (18).

For the age groups younger than two years, greater than 80 percent of the estimated intake was provided by two food categories, baked goods and frozen dairy desserts. Fewer than four manufacturers reported adding tannic acid to any baked goods. Six manufacturers reported adding tannic acid to frozen dairy desserts. There were no reports of the addition of tannic acid to baby food or infant formula products (8). The Select Committee believes the intakes in Table II are greatly overestimated.

The NRC subcommittee considered that the poundage of a GRAS substance reported used by the surveyed manufacturers may represent only 60 percent of the actual usage (8). Recalculated to 100 percent, 791,838 kg of food grade tannic acid was used in 1970. On the basis of a 205 million population the per capita daily consumption was about 10 mg. However, much of this was used as a processing aid. Assuming the FEMA survey covered tannic acid's use as a flavoring agent (1,701 kg) and the NRC respondents reported the use as a processing aid (473,402 kg), the per capita daily intake of added tannic acid as a flavoring agent, recalculated to 100 percent as above, was about 0.04 mg.

The Joint FAO/WHO Expert Committee on Food Additives in 1970 proposed a temporary acceptable daily intake of tannic acid for man of 0.6 mg per kg for Peruvian tara (essentially polydigalloyl esters of quinic acid) or 0.3 mg per kg for Turkish Aleppo, Chinese tara, and Sicilian sumac (all consist of polydigalloyl esters of glucose) (11,19). They further commented that some tannins occurring in natural foodstuffs, such as tea and coffee, are not toxic, and that early studies on commercial tannin showing hepatotoxicity bear little relevance to the evaluation of food grade material.
IV. BIOLOGICAL STUDIES

Tannic acid is considered an hepatotoxic and cirrhotic agent capable of causing severe and even fatal disease at concentrations formerly used in the topical treatment of burns or in certain tannic acid barium enemas (4). With a few exceptions, data on toxicity resulting from routes of administration other than oral are not relevant to an evaluation of possible hazards from the food uses of tannic acid.

Absorption, metabolism and excretion

Korpassy et al. (20) in 1951 tested the absorption of a single dose of tannic acid (U.S.P. Hendon, London) administered by gavage in aqueous solutions to rabbits (0.5 to 2 g per kg) and dogs (0.3 to 2.3 g per kg). The animals were fasted 24 hours prior to the experiment. A dose related increase in plasma phenolic group concentration was noted; however, the test method (21) detected phenolic groups and was not specific for unhydrolyzed tannic acid. Two of 100 dogs died during the experiment; one died of unknown causes five hours after receiving 415 mg tannic acid per kg, but the second, that received 830 mg per kg, showed extensive acinocentralis (centrolobular) necrosis of the liver. Necropsies were performed on several of the dogs and rabbits but no results were reported.

In 1962, Dollahite et al. (22) measured serum levels of tannic acid of 52 to 230 μg per ml by a nonspecific test for phenolic groups (21) after gastric intubation of aqueous tannic acid (reagent grade) solutions at 2 to 8 g per kg to rabbits. At 24 hours after intubation, rabbits receiving more than 4 g per kg had died; "tannic acid" had been eliminated from the serum of the animals receiving up to 4 g per kg. Goats and sheep had "tannic acid" blood levels of 75 to 160 μg per ml within one hour after receiving by the same route a 2 g per kg dose; the blood tested negative at 48 hours.

Most reports of the absorption of tannic acid have relied on nonspecific identification of phenolic substances in blood sera. However, Harris et al. (23) in 1966 used thin layer chromatography to identify tannic acid in the blood serum of Long-Evans rats (200 to 250 g) in a study of colonic absorption. Aqueous tannic acid (N.F.) enemas were administered to the animals. Increased amounts of tannic acid were found in hepatic portal vein blood as the concentration and contact time were increased, but no tannic acid was absorbed from a 1 percent solution in the colon from one minute to one hour. There was a trace (approximately 25 μg per ml) of tannic acid in the blood one minute after a 2 percent tannic acid enema was given.
Kessler and Blumenberg (24) using paper chromatography have demonstrated the breakdown of tannic acid by the gastrointestinal juices of guinea pigs to gallic acid and glucose. These authors, in another report, were unable to demonstrate intact tannic acid in rabbit blood serum after intubation of tannic acid solutions (25).

A major metabolite in the urine of rabbits, rats, and chickens ingesting tannic acid or gallic acid was identified as 4-0-methyl gallic acid (26, 27). The increases in urinary excretion of 3,4-dihydroxyphenolic and phenolic methoxy substances by man after ingestion of 1 g tannic acid (not described) were interpreted as due to metabolic products of tannic acid (28).

The addition of methionine and choline as a methyl-group source to a diet of high tannin grain sorghum improved feed efficiency and corrected growth depression and body weight loss in chicks and laying hens (29, 30). Jambunathan and Mertz (31) demonstrated an inverse relationship between rat growth rate and the polyphenolic compound content of sorghum varieties.

Mitjavila et al. (32) studied the absorption of glucose, butyric acid and methionine, using in vivo perfusion techniques in the presence of gallo-tannic acid in the mouse intestine. Statistical analysis of the perfusion data showed that 0.005 to 0.25 percent tannic acid produced a marked reduction in the absorption of water, glucose and methionine, and a less significant reduction in butyric acid absorption. Concurrent histological studies showed edema of the intestinal mucosal villi with shrinkage of the layer of epithelial cells when 0.25 percent tannic acid was tested. This was attributed to denaturation of the intestinal epithelial cells and to reduction in the capability of these cells to actively transfer glucose and methionine through the wall.

Acute toxicity

Korpassy et al. (33) in 1950 administered tannic acid solutions (2.5, 5.0, and 10.0 percent) by gavage two or four times daily for three days to rats (0.75 to 3.0 g per kg), and for one or three days to rabbits (0.67 to 2.7 g per kg), and dogs (0.9 to 3.6 g per kg). Autopsies of animals that died as a result of the test doses and of those sacrificed three days after the last dose showed varying degrees of hepatic necrosis in a few animals receiving 2.5 percent tannic acid, in most of those receiving 5.0 percent and in all receiving 10.0 percent solutions.

In mice, the oral I.D50 of tannic acid (Merck, U.S.P., Chinese nut-gall) administered by stomach tube as a 10 percent aqueous solution was between 3 and 4 g per kg body weight (34). Rats were more resistant than mice to tannic acid, and doses three to four times larger were required to produce toxic signs. The authors reported that the toxicity of gallic acid was less than that of tannic acid in mice, and "large doses" of gallic acid did not produce the signs of tannic acid toxicity. Other workers have noted
the differences in toxic effects of tannic acid and gallic acid in rats after intraperitoneal administration (23). Doses of 40 mg tannic acid per kg produced no hepatohistologic damage in five animals; doses of 60 mg per kg produced hepatohistologic damage in each of five other rats. Gallic acid had no hepatotoxic effects; even at a lethal dose of 2000 mg per kg only renal damage was noted.

Boyd et al. (35) reported in 1965 on the toxicity of tannic acid (Fisher Certified) [probably mistakenly referred to as 3-galloyl gallic acid by the authors] in young male albino Wistar rats weighing 125 to 200 g. Doses of 2.0 to 8.0 g per kg were administered by intragastric cannula in solutions of 20 ml per kg body weight. The oral LD50 was 2.26 (S.E. 0.083) g per kg with a mean interval between dose and death of 38 hours. Doses of 4.5 to 8 g per kg were fatal within six hours. Drowsiness, pallor, cyanosis and diarrhea were the most common signs of intoxication. Anorexia and oligo-dipsia also were evident. Death was preceded by tremors and convulsions and finally respiratory failure. A temporary fulminating gastroenteritis followed oral administration but was gone at three days. Hepatic necrosis and nephritis were evident in animals surviving two to four days. The spleen showed hyperplasia as did the adrenal cortex. Spermatogenesis was stimulated in animals dying at two or three days, and those surviving for several weeks had significantly heavier testes.

Těxl and Konečný (36) performed experiments utilizing oral, subcutaneous and rectal treatments of Wistar rats with various tannic acid (Ac. tannicum-Lachema, PhB-2) concentrations. The sulfobromophthalein dye test was used to observe liver function. Oral doses of 1 g per kg of tannic acid caused no damage to the intestinal tract and no impairment of liver function. The author considered this proof that the undamaged digestive tract is impermeable to tannic acid. Doses of 2 and 3 g per kg produced significant liver function impairment, centrolobular necrosis and loss of glycogen, and inflammatory changes and ulceration of the gastric mucosa. After gallic acid was administered subcutaneously (1 g per kg) or rectally (1.5 g per kg) no impairment of liver function could be detected, liver histology appeared normal, and the rectal mucosa was unaffected.

Mitjavila et al. (37) in 1971 studied the effect of tannic acid on the phagocytotic activity of the reticuloendothelial system of the liver in male Wistar rats. In these experiments tannic acid (Aleppo) was administered intravenously or by gastric intubation. The results of the intravenous and oral experiments were similar. The hepatotoxicity of intravenously administered tannic acid (20 mg per kg) was similar to that seen after intragastric administration of higher doses (2 g per kg). Slightly less bile was excreted during the first hour, sulfobromophthalein elimination was significantly reduced, and Kupffer cell activity was normal during the first 24 hours but increased by 82 and 75 percent during 48 and 72 hours after injection, respectively. Parenchymal clearance decreased about 23 percent after 24 hours and the decrease persisted after 48 hours. The level of hepatic triglycerides was significantly increased 72 hours after injection.
Dollahite et al. (22) gastrically intubated rabbits with aqueous solutions of tannic acid (unidentified), gallic acid and pyrogallol; the single dose $LD_{50}$s for these compounds in rabbits were 5.0, 5.0, and 1.6 g per kg, respectively.

**Short-term studies**

Peaslee and Einhellig (38) found the age and weight of mice affected the animals' response to dietary tannic acid. They fed groups of six to eight young male albino mice a diet containing 5 or 8 percent tannic acid (Turkish nutgall, technical grade) in ground laboratory chow for periods of three to eight weeks. Daily tannic acid intake for the two levels was about 16 and 25 g per kg body weight averaged over the test period. Mice starting the test with body weights less than 12 g and fed an 8 percent tannic acid diet continued in depressed growth to sacrifice, exhibited a significant decrease in pituitary gland weight and had a significant increase in pituitary melanocyte stimulating hormone activity. This was postulated by the authors to be evidence of the toxic effect of prolonged intake of tannic acid by young animals.

Handler and Baker (39) found no evidence of hepatic necrosis in Vanderbilt rats given tannic acid (unidentified) at levels of 1 and 2 percent (about 1 to 3 g per kg) in the diet of pair-fed rats over a period of 90 days. No gross or histological signs of liver damage were observed.

Racela et al. (40) studied reagent grade gallotannic acid in 60 female Fisher-344 rats. The rats were sacrificed from one to three weeks after a single subcutaneous injection of 700 or 1200 mg tannic acid per kg body weight. Complete autopsies revealed an early and promptly reversible form of nucleolar macrosegregation or "capping." Iron-ribonucleic acid complexes were presented as intranuclear inclusions which disappeared gradually over a two-week period from the centrolobular areas. A transient, marked increase in fat in the liver cells diminished to a slight increase after 72 hours. Cytoplasmic degeneration was reflected in irregular eosinophilic bodies, some periodic acid Schiff positive, that were free of glycogen. Focal cytoplasmic necrosis was most prominent in central and midlobular zones. Most affected hepatocytes were within normal limits by one week after tannic acid treatment.

Glick and Joslyn (41, 42) fed 50 g weanling Long-Evans rats ad libitum a basal 20 percent casein diet containing 0, 4, and 8 percent tannic acid (analytical reagent grade gallotannic acid, J.T. Baker). At the 8 percent tannic acid level (1.8 g per kg) nine of ten animals died in four to seven days as did eight of ten pair-fed controls receiving a tannic acid-free basal diet. Growth was depressed because of decreased food intake as compared to ad libitum fed controls, but no deaths occurred at the 4 percent tannic acid level. Mature rats (200 g) on the 8 percent tannic acid diet (0.9 g per kg) had no mortality but gained less weight than pair-fed controls. The growth of 130 g rats on 5 percent tannic acid in a 40 percent casein diet was
improved as compared to the basal 20 percent casein diet and was equal to that of pair-fed controls; protein absorption was increased. Methionine or choline added to the basal diet containing 5 percent tannic acid produced no significant improvement in growth rate. The authors noted high fecal nitrogen losses in rats fed tannic acid. The toxicity of tannic acid appeared to be inversely related to age and weight of the animals.

Squillaci and DiMaggio (43) administered tannic acid (1 g per kg) in water daily for 40 days by stomach tube to male rabbits (2 kg) fasted for 12 hours. The general condition of the animals was good for the first 20 days. Later the body weight decreased and albumin showed in the urine at the end of the experimental period. Histological examination revealed certain toxic effects including degeneration of hepatic cells closest to the centrolobular veins, and areas of scaling of the stomach and small intestine mucosal epithelium.

The Joint FAO/WHO Expert Committee on Food Additives in 1970 reported studies of rats and dogs given various dietary levels of hydrolyzable tannins (19). The rats received 0, 8, 80, or 800 mg per kg body weight of Aleppo or tara tannin in the diet for 12 weeks without significant changes in body weight or food intake and utilization. No liver or kidney abnormalities attributed to the tannins were seen at necropsy. Similar studies with Chinese, Sicilian sumac and Douglas fir tannins produced comparable results. Dogs were given 0.0, 0.117, 0.125, 0.234, and 0.25 percent tannin in their diet as chewing gum (containing 0.5 percent Peruvian tara tannin) for two years with no ill effects. Behavior, food consumption, hematology, organ function tests, organ weights, gross and microscopic pathology were within normal limits.

**Long-term studies**

A report of the Joint FAO/WHO Expert Committee on Food Additives (19) detailed a long-term study in which 100 rats were fed Peruvian tara tannin as 0.0, 0.117, 0.125, 0.234, or 0.25 percent of their diet. In weanling rats, the highest dosage was about 0.3 g per kg body weight. The tannin was incorporated in a chewing gum at a level of 0.5 percent and then added to the diet. No deleterious effects were seen by the usual criteria of growth, food consumption, and gross and microscopic pathology. In a three-generation study, two litters per generation, groups of 20 male and 20 female rats were fed Peruvian tara tannin at levels of 0.0, 0.058, 0.117, and 0.234 percent. The young at weaning fed the highest level of tannin had lower body weights, a feature not observed at lower levels of tannin ingested. Fertility, gestation, viability and lactation were not affected at any feeding levels.

Korpassy et al. (44) administered aqueous solutions of tannic acid (U.S.P.) to rats by oral intubation before meals. The animals were dosed (initially, 0.27 g per kg) four times each week with 4 ml of 1.0 percent
tannic acid. The tannic acid concentration was increased by 1.0 percent every other month to a maximum of 5.0 percent (1 g per kg). Of 20 rats in this group, seven survived the 390 days of treatment. A second group of 40 rats was dosed once each week with 4 ml of 3 percent tannic acid for one month (initially 0.8 g per kg) and 5 percent for the remainder of the testing period; twenty-eight survived 300 days and four survived 500 days. Autopsies were performed and tissues examined microscopically. The enlarged livers and spleens showed extensive damage in two animals that died and one that was sacrificed during the experiment. There were numerous dividing myelogenous cells in the bone marrow in all of the animals. The kidneys, adrenals and ovaries showed a slight degree of myelogenous leucocytic infiltration. The histological appearance was suggestive of marked myelogenous hyperplasia. The hematopoietic and reticuloendothelial systems showed an activity indicative of response to stimulation of the active mesenchyme.

Weanling male and female albino CF mice were raised on a diet of ground laboratory chow and 8 percent tannic acid (about 12 to 25 g per kg) of technical grade, Turkish nutgall. In one experiment these animals were maintained on the tannic acid diet through two breeding cycles, and they produced litters of smaller number, slower growth rate, and lower body weight plateau (45). When female mice were removed from the tannic acid diet prior to breeding by control males, there were no significant differences between test and control litter weights or animals per litter. The authors suggested that the protein binding activity of tannic acid may influence hormonal activity. No data on feed intake were reported.

Special studies

As defined in the background section of this report, food grade tannic acid is a hydrolyzable gallotannin. Some data on condensed tannins are included in this report for comparative purposes, but the health aspects of condensed tannins are not reviewed.

The work of Korpassy (46-49) indicated that repeated subcutaneous administration of tannic acid (USP) to rats produced hemorrhagic gastric erosions, skin necrosis and ulcers, and hepatic tumors arising in sites of nodulār cirrhosis and irregular bile duct proliferation. Doses of 150 to 200 mg per kg body weight administered every fifth day resulted in 30 to 50 percent survival in rats at 200 days and 5 to 15 percent survival for 300 days. More than half of the animals surviving 100 days developed hepatomas and/or cholangiomas, usually multiple and benign, although some histological patterns suggested low-grade malignancy. No metastases involving liver cancer were produced by tannic acid treatment and local carcinogenic effects were not observed associated with the skin ulcers resulting from subcutaneous injections. In reviewing the work in his laboratory on the oral administration of tannic acid to rats, Korpassy has reported no liver
tumors in tannic acid treated animals and no gastric erosions after treatment with 2 to 5 percent aqueous tannic acid solutions. Precirrhotic changes were observed in livers of rats after 180 days of treatment per os but only if the dose exceeded the effective parenteral dose by several times.

Kirby (50) studied tumor induction in groups of 10 rats or mice after subcutaneous injection of tannic acid (B. D. H.), extracts containing condensed tannins, or extracts containing hydrolyzable gallotannins from myrobalans (Terminalia chebula Retz.), chestnut (Castanea sativa Mill.), and valonea (Quercus aegilops L.). Twelve equal weekly doses delivered a total of 350 mg of tannin extract or tannic acid per kg to rats and 750 mg per kg to mice. Sarcomas appeared at the injection site of condensed tannin extracts in rats after one year but did not develop at the injection sites of tannic acid or myrobalan extract, the only hydrolyzable gallotannins tested in the rat. No liver damage or other observable effects were produced in the rat by these hydrolyzable gallotannins. In mice, tannic acid and extracts of myrobalans, chestnut and valonea produced no tumors at the site of injection but liver tumors were described in each of these groups.

Several reports, in addition to the long-term feeding studies reviewed by the Joint FAO/WHO Expert Committee on Food Additives (19), are negative with respect to carcinogenic effects after parenteral or oral administration of tannic acid. Bichel and Bach (51) injected intramuscularly a group of 30 male and 30 female C3HA mice with nutgall tannic acid (0.75 mg per kg) every second week for 12 months. The tannic acid was described as equivalent to tanninum (Ph. Nord.), acidum tannicum (Brit. 32) and to tannic acid (USA 47). It was delivered in a mixture of 1 mg cyanocobalamin and 1.5 mg tannic acid in 2 percent aluminum monostearate gel in sesame oil. Equal numbers of mice received the tannic acid preparation without cyanocobalamin and a third group received saline injections. The experiment was terminated six months after the last injection. There were no tumors at the injection sites and histological examination of the liver revealed no differences in the three groups. Perissinotto and Lombardi (52) injected 80 rats subcutaneously with 1 to 2 percent aqueous solution, delivering 100 to 200 mg of tannic acid (not described) per kilogram body weight every five days and reported no evidence of liver tumors after 250 days.

Blumenberg and Kessler (25) administered tannic acid (1000 mg per kg body weight of DAB 6) by gavage daily for 231 days to male Wistar rats and noted no hepatonecrosis. Konstantinov and Ivanov (53) injected subcutaneously 200 mg tannic acid (not described) per kg to chickens weekly for 36 weeks. Intranuclear inclusions in parenchymal cells of the liver and kidney tubules were noted 24 hours after the initial treatment but no hepatomas or cholangiomas developed during the test period. The cellular inclusions indicated repair of the liver cells rather than evidence of carcinogenic changes.
Weinberg et al. (54) studied the influence of drug treatments (including tannic acid) given to female rats during gestation on the toxicity of drugs administered to the newborn. There was no influence on the susceptibility of the newborn rats (12 to 24 hr) to lethal doses of tannic acid when the dam was treated before or during gestation. The study showed that younger rats died earlier mainly because of their inability to tolerate the gastrointestinal necrosis that tannic acid induced.

Food grade tannic acid (55) was not mutagenic when tested by in vitro microbial assays with and without the addition of mammalian (mice, rats, and monkeys) metabolic activation preparations (liver, lung and testes) (56). The indicator organisms used were Saccharomyces cerevisiae D4 and Salmonella typhimurium TA-1535, TA-1537 and TA-1538.

Teratologic evaluation of food grade tannic acid (55) was conducted in mice and rats (57). Administration of up to 135 mg per kg body weight of tannic acid to pregnant mice and up to 180 mg per kg body weight of tannic acid to pregnant rats for 10 consecutive days (day 6 through 15 of gestation) had no clearly discernible effect on nidation or on maternal or fetal survival. The number of abnormalities seen in either soft or skeletal tissues of the test groups did not differ from the number occurring spontaneously in the sham-treated controls. Food grade tannic acid (55) displayed no teratogenicity in the chick embryo test (58).

Johnston et al. (59) reported the case history of one patient who was allergic to both tannic acid and gallic acid.

V. OPINION

The Select Committee considers only hydrolyzable gallotannins to be within the definition of tannic acid. Tannic acid yields gallic acid and either glucose or quinic acid as hydrolysis products. These hydrolysis products are consumed as naturally occurring constituents of many foods. The total dietary intake of added tannic acid resulting from its use as a flavoring agent or processing aid can be inferred to be very small. The level of addition of tannic acid to various foods is about 100 ppm or less. Thus, the potential level of consumer exposure is orders of magnitude below the lowest levels for which adverse biological effects are reported to occur after oral administration of tannic acid to experimental animals.

While the evidence seems clear that food grade tannic acid at concentrations reportedly added to foods is hydrolyzed prior to absorption from the small intestine, the extent and nature of its absorption have not been established.
for man. In experimental animals, intact tannic acid is apparently absorbed only after mucosal damage caused by large doses of concentrated aqueous tannic acid solutions intubated into empty stomachs or by artificially prolonged exposure of the mucosa to these solutions. Data available to the Select Committee contain no evidence that tannic acid as currently used in foods can induce mucosal damage. However, toxic effects following parenteral administration to animals suggest that additional studies should be made related to the absorption of tannic acid at dietary levels by individuals with gastrointestinal disorders.

Some investigators have reported that daily oral administration of tannic acid to experimental animals by stomach tube for periods of months may cause damage to the liver and other organs similar to that seen after parenteral administration of hydrolyzable tannins to experimental animals, and to that reported historically in man following topical treatment of burns with tannic acid. None of these effects is reported, however, in experimental animals receiving tannic acid as a component of their ration, even at levels of several percent of the diet. Nevertheless, the reported hepatocarcinogenic effect of parenterally injected hydrolyzable gallo-tannins is of concern to the Select Committee owing, in part, to the lack of uniformity of composition and sources of the preparations used in the tests available in the literature for evaluation. While the evidence available to the Select Committee does not demonstrate hazard of carcinogenicity inherent in food grade tannic acid, research is needed to answer the question of why there are such extreme differences between the results of parenterally and orally administered hydrolyzable tannins in rodents.

There is no evidence of a clear and present danger to the public health occasioned by the current use of dietary tannic acid as a food ingredient. The Select Committee, therefore, in the light of the foregoing concludes that:

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


VII. SCIENTISTS CONTRIBUTING TO THIS REPORT

1. Members of the Select Committee on GRAS Substances:

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

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

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

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

John R. McCoy, V.M.D., Professor of Comparative Pathology, New Jersey College of Medicine and Dentistry, Rutgers Medical School, New Brunswick, N.J.

Sanford A. Miller, Ph.D., Professor of Nutritional Biochemistry, Massachusetts Institute of Technology, Cambridge, Mass.

Gabriel L. Plaa, Ph.D., Professor and Chairman, Department of Pharmacology, University of Montreal Faculty of Medicine, Montreal, Canada.

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

Ralph G.H. Siu, Ph.D., Consultant, Washington, D.C.

John L. Wood, Ph.D., Distinguished Service Professor, Department of Biochemistry, University of Tennessee Medical Units, Memphis, Tenn.

George W. Irving, Jr., Ph.D., (Chairman), Life Sciences Research Office, Federation of American Societies for Experimental Biology, Bethesda, Md.
2. LSRO staff:

Kenneth D. Fisher, Ph.D., Director
Frederic R. Senti, Ph.D., Associate Director
C. Jelleff Carr, Ph.D., Director Emeritus
Richard G. Allison, Ph.D., Staff Scientist
Andrew F. Freeman, Senior Staff Scientist
John M. Talbot, M.D., Senior Medical Consultant
Michael J. Wade, Ph.D., Staff Scientist

The Select Committee expresses its appreciation to the following organizations and institutions who contributed information and data:

F.H. Kratzer, Ph.D., Department of Avian Sciences, University of California, Davis, Calif. 95616

Mr. Stephen Lirot, Nestle Company, Inc., 100 Bloomingdale Road, White Plains, N.Y. 10605

Report submitted by:

November 7, 1977

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