DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Washington, DC 20204

JUL 31 1998

Rec'd 8/5/98

Douglas D. Lazarus, Ph.D.
17 Winter Street
Watertown, Massachusetts 02172

Dear Dr. Lazarus:

This is in response to your letter to the Food and Drug Administration (FDA) dated June 18, 1998, concerning the safety of pokeweed mitogen used as an ingredient in dietary supplements. Your letter responded to letters from FDA, dated September 24, 1997 and March 31, 1998 to Mr. Sam Berkowitz of Advanced Plant Pharmaceuticals, Inc. and you, respectively, regarding a submission to FDA pursuant to 21 U.S.C. 350b (section 413 of the Federal Food, Drug, and Cosmetic Act (the Act)).

In our letter to Mr. Berkowitz, FDA stated that it fundamentally disagreed with a determination made by him that a dietary supplement containing a mixture of lectins from the pokeweed plant (*Phytolacca americana)* will reasonably be expected to be safe. FDA tentatively concluded that pokeweed was a well-characterized poisonous plant and that toxicity to humans and animals was associated with exposure to all parts of the pokeweed plant, and that the lectins contained in pokeweed were believed to be one of primary biochemical contributors to pokeweed toxicity. FDA advised Mr. Berkowitz that his submission did not meet the requirements in 21 U.S.C. 350b and that introduction of this product into interstate commerce was prohibited under 21 U.S.C. 331(v) and that a dietary supplement containing his proposed new dietary ingredient would be subject to regulatory action pursuant to 21 U.S.C. 342(f)(1)(B).

In a letter to you dated March 31, 1998, FDA stated that it had considered the information presented in your letter and was not persuaded to change its tentative conclusion that pokeweed mitogen, when used under the conditions recommended or suggested in the labeling of the dietary supplement, will not reasonably be expected to be safe. After careful consideration of the information in your most recent letter, the agency is not persuaded to change its position as set forth in our letter of March 31, 1998. Therefore, the introduction or delivery for introduction of a dietary supplement containing pokeweed mitogen into interstate commerce is prohibited under 21 U.S.C. 331(v) and a dietary supplement containing this proposed new dietary ingredient would be subject to regulatory action pursuant to 21 U.S.C. 342(f)(1)(B).

955-0316

SUP3

Please contact us if you have any questions concerning this matter.

Sincerely,

James T. Tanner, Ph.D.
Acting Director
Division of Programs and Enforcement Policy
Office of Special Nutritionals
Center for Food Safety
and Applied Nutrition

Copy:

Mr. David Berkowitz
Advanced Plant Pharmaceuticals, Inc.
17 John St., 3rd Floor
New York, NY 10038

59797

June 18, 1998

RECEIVED 6/19/98

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Dear Dr. Tanner:

In your letter of March 31, 1998, the FDA tentatively concluded that a dietary supplement containing pokeweed mitogen would not be expected to be safe. The purpose of this submission is to present 1) toxicology from case histories of pokeweed plant poisoning, 2) scientific studies of pokeweed mitogen and 3) a description of the proposed dietary supplement for reconsideration of pokeweed mitogen as being safe for consumption. Included are the references which I cite in this overview of pokeweed mitogen and human exposure to the pokeweed plant.

Pokeweed poisoning

Pokeweed, *Phytolacca americana*, is a native perrenial shrub-like plant, common in the eastern United States. It has been used as a food source for centuries (1-3). However, the leaves must be prepared properly, boiling twice and discarding the first boiling, which removes toxins present in the plant (1, 2). Without proper preparation, pokeweed poisoning develops. The symptoms of pokeweed poisoning are severe gastrointestinal disturbances, with vomiting, abdominal cramps, diarrhea, sweating, confusion, tremor, weakness, incontinence and syncope (2). Recovery is usually complete by the next day (2), but deaths have occurred due to the pokeweed plant (1, 4).

The toxins responsible for pokeweed poisoning are present in all parts of the plant (1, 2, 4). These include saponins and two alkaloids, phytolaccine and phytolaccotoxin (1, 4). Pokeweed mitogen, a glycoprotein with mitogenic activity, is also considered to contribute to the toxicity of this plant (4). However, such purported toxicity is mild, since the only effect attributed to pokeweed mitogen is promotion of white blood cell division, causing plasmacytosis, and

stimulation of the production of interferon (4). The plasmacytosis is only of diagnostic significance (4, 5).

In the 1960s, Barker and Farnes studied the blood effects of pokeweed closely (6, 7). This group found that ingestion of pokeweed was not necessary. Simple exposure of cuts or abrasions to pokeweed berry juice caused plasmacytosis (6). Exposure to pokeweed berry juice did not cause any symptoms or clinical signs other than the plasmacytosis described (7). The blood changes resolved on their own, with no long term cell lines induced by exposure to pokeweed mitogen (6).

In summary, the small organic molecules in pokeweed cause the severe gastrointestinal response to pokeweed ingestion (1, 2, 4). Pokeweed mitogen has not been shown to cause any of the gastrointestinal symptoms characteristic of pokeweed poisoning (4-7).

Pokeweed mitogen

The term pokeweed mitogen refers to a mixture of 5 different proteins, all of which are lectins, i.e., a class of proteins which bind to carbohydrate (8, 9). Pokeweed lectins all bind to N-acetyl glucosamine (10-12).

The structures of pokeweed mitogen lectins are only recently being described. The pokeweed lectins whose amino acid sequence have been determined are similar to that of wheat germ agglutinin (11, 12). Wheat germ agglutinin also binds to the same ligand as pokeweed mitogen, N-acetyl glucosamine (13). Wheat germ agglutinin is present in wheat germ, a food which can be purchased in most health food stores.

Pokeweed mitogen is present in all parts of the plant - roots, leaves, berry juice, etc. The plant part used for purification of pokeweed mitogen as described in the literature was the root (14). Processing involves grinding the root in phosphate-buffered saline, heating to 75°C to coagulate large proteins, centrifugation to remove the coagulated proteins, further precipitation of protein with trichloroacetic acid and, after removal of the trichloroacetic acid by dialysis, passing over a calcium phosphate column (14). This results in a preparation from which the dietary supplement described below would be produced.

Experimental research with pokeweed mitogen

Pokeweed mitogen has been used experimentally in two ways. One is as a laboratory tool for the *in vitro* stimulation of lymphocytes, and will not be considered further here. *In vivo* investigation, i.e., direct administration of pokeweed mitogen to rodents, has focused on three areas:

I. Studies carried out in the National Institute of Mental Health used pokeweed mitogen as a model of brain injury. Administration of pokeweed mitogen to mice intraperitoneally as a single dose of 25mg/kg was followed by elevated circulating concentrations of the neurotransmitter metabolites quinolinic acid, kynurenic acid and kynurenine (15). Early in the course of this work, the source of the metabolites was believed to be the brain, with the

increase reflecting brain injury. Subsequent work with this model found that the source of the metabolites was immune cells from the peripheral circulation, not the brain, and that no brain injury is caused by pokeweed mitogen (16).

II. Because pokeweed mitogen stimulates T lymphocytes and B lymphocytes, effects of pokeweed mitogen on the immune function of mice was investigated. In a study of effects on the spleen, pokeweed mitogen was given as a single i.v. dose of 160mg/kg. Spleen weight and plasma cell number increased after pokeweed mitogen treatment, but plasmacytosis did not develop (17). There were no deaths following this dose (17).

An additional study in which mice were administered pokeweed mitogen examined the spleen, lymph nodes, liver, lungs and thymus (18). In that work, the highest treatment was a single i.p. dose of 55mg/kg. Spleen weights and spleen lymphocyte colonies increased. No unusual spleen cellular components were detected. Lymph nodes were enlarged. Liver, lung and thymus weights did not change. Spleens and lungs showed enhanced erythrophagocytosis following pokeweed mitogen. Carbon clearance from the blood increased. As also occurs with humans, plasmacytosis developed.

Because no systemic effects or mortality were found, the conclusions of the authors were that no toxic effects followed pokeweed mitogen administration at the doses used (18).

III. A series of studies examined the effects of pokeweed mitogen on body weight, food intake and blood glucose concentrations in mice (19). Circulating interleukin-6 (IL-6), but not tumor necrosis factor-α, increased following pokeweed mitogen administration. In a group which received pokeweed mitogen at a dose of 5mg/kg i.p. every 2 days, 3 of 5 mice starved to death in 3 days. There was no diarrhea or other overt sign of gastrointestinal disturbance in mice given 5mg/kg. When a dose of 3mg/kg was given i.p. every 2 days, the mice lost 10% of their body weight, but all survived the duration of the study - 16 days - without any gastrointestinal disturbances apparent at any time or gross pathology apparent on necropsy.

The studies outlined above used doses ranging from 3mg/kg to 160mg/kg. The high dose - 160mg/kg - did not cause any lethality after a single dose. The lowest dose - 3mg/kg - did not cause any death or apparent organ disfunction despite receiving this dose for 16 days.

While IL-6 is known for its role in inflammatory conditions, such as rheumatoid arthritis, anti-inflammatory effects were also described (20, 21). Indeed, a single pokeweed mitogen administration (5mg/kg i.p.) to mice prior to the induction of delayed type hypersensitivity in an ear-swelling model suppressed swelling 80% (Figure 1). The anti-inflammatory activity of IL-6 was likely to be the underlying cause of this protection. IL-6 is a mitogenic factor for immune system-derived cancers, such as lymphoma (22). However, no increased risk factor for cancer has been noted in conditions associated with chronic elevated circulating IL-6 concentrations.

Product description

I propose to market a dietary supplement for sale which contains pokeweed mitogen. The function claim of this dietary supplement would be to stabilize and maintain present body weight.

This product would be called Phytolacca extract, and be made available in a 150mg tablet form. Each tablet would contain 2mg of pokeweed mitogen. The rest of the tablet would be composed of sucrose, cellulose and magnesium stearate excipients. With a suggested dose of up to 3 tablets a day, there would be no more than 6mg of pokeweed mitogen taken per day. At this dose, a 38kg woman would ingest up to 0.16mg/kg. This is 5% of the lowest dose administered to mice in work described in the scientific literature - a dose which was not associated with observations of toxicity in animals.

Because 2.5mg/kg does not induce hypoglycemia in mice (Figure 2), the maximum recommended dosage of 6mg per day, which for a 38kg woman is 6% of the 2.5mg/kg dose, is not expected to cause hypoglycemia.

Phytolacca extract label

The label for Phytolacca extract would state:

1) plant source of Phytolacca extract as *Phytolacca americana*
2) excipient ingredients and quantity of Phytolacca extract per tablet in milligrams
3) Phytolacca extract may help stabilize and maintain present body weight
4) recommended dosage of not more than 3 tablets per day
5) for adult use only
6) company name, address and phone number
7) date of production and lot number
8) instructions to store at room temperature for not more than 9 months from date of production (based on personal observations of bioactivity in mice)

I look forward to your reconsideration of my proposal for the sale of Phytolacca extract as a dietary supplement. Thank you very much for your attention to this matter.

Sincerely,

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References

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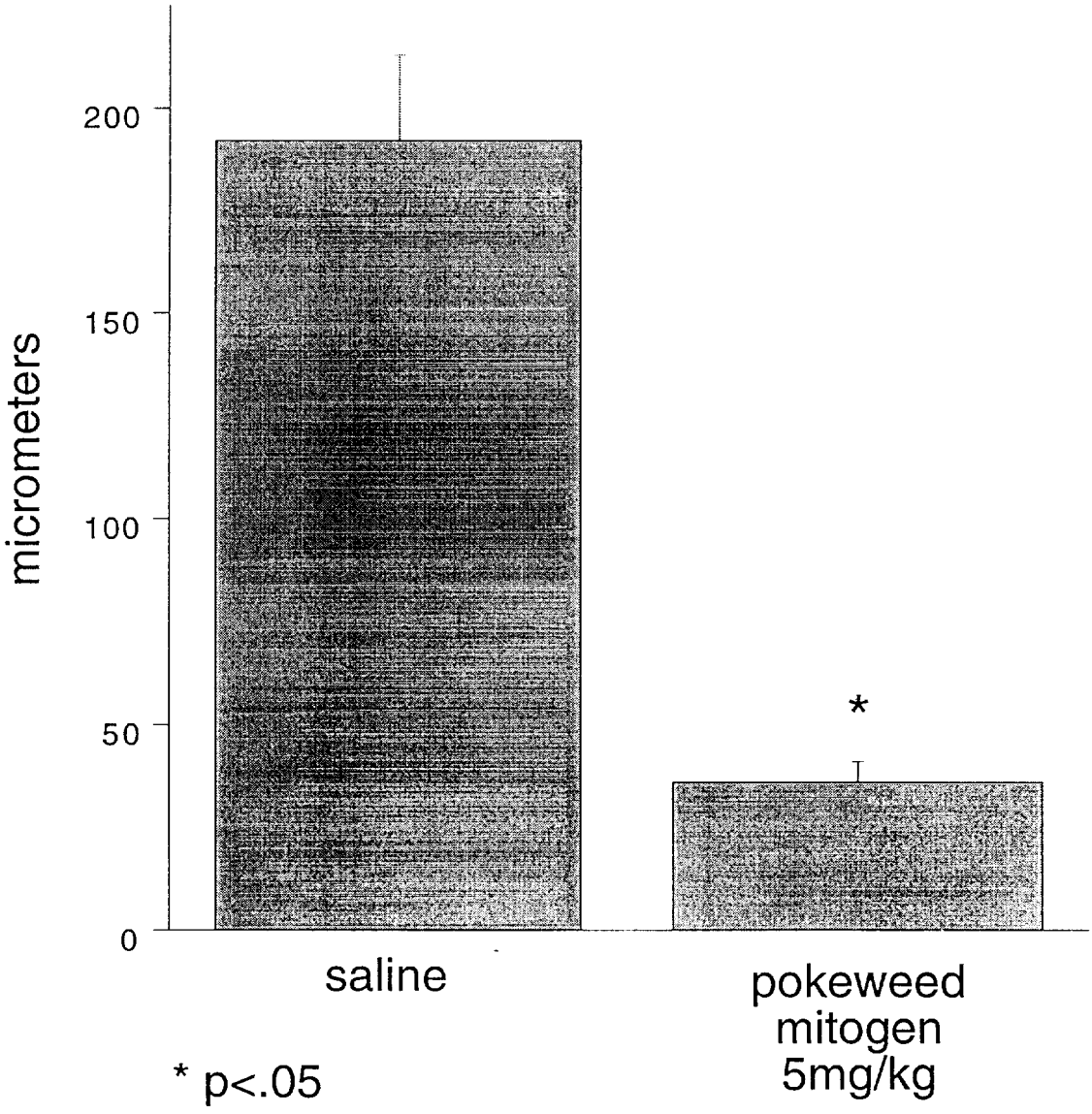
Figure 1. Ear swelling in mice made sensitive to dinitrofluorobenzene



* p<.05

Figure 2. Circulating glucose concentrations in mice given pokeweed mitogen