mole of methyl laurate with 1.05 moles of diethanolamine..	
Triethanolamine.....	Do.
Ethylene glycol monobutyl ether.....	Not to exceed 1 ppm.
Oleic acid conforming with Sec. 172.860 of this chapter.	Do.
Tetrapotassium pyrophosphate.....	Not to exceed 0.3 ppm.
Monoethanolamine.....	Do.
Ethylene dichloride.....	Not to exceed 0.2 ppm.
Tetrasodium ethylenediaminetetraacetate...	Not to exceed 0.1 ppm.

(b) The chemicals are used in amounts not in excess of the minimum required to accomplish their intended effect.

(c) The use of the chemicals listed under paragraphs (a)(1), (a)(2), and (a)(4) is followed by rinsing with potable water to remove, to the extent possible, residues of the chemicals.

(d) To assure safe use of the additive:

(1) The label and labeling of the additive container shall bear, in addition to the other information required by the act, the name of the additive or a statement of its composition.

(2) The label or labeling of the additive container shall bear adequate use directions to assure use in compliance with all provisions of this section.

[42 FR 14526, Mar. 15, 1977, as amended at 42 FR 29856, June 10, 1977; 42 FR 32229, June 24, 1977; 43 FR 54926, Nov. 24, 1978; 61 FR 46376, 46377, Sept. 3, 1996; 63 FR 7069, Feb. 12, 1998]

Sec. 173.320 Chemicals for controlling microorganisms in cane-sugar and beet-sugar mills.

Agents for controlling microorganisms in cane-sugar and beet-sugar mills may be safely used in accordance with the following conditions:

(a) They are used in the control of microorganisms in cane-sugar and/or beet-sugar mills as specified in paragraph (b) of this section.

(b) They are applied to the sugar mill grinding, crusher, and/or diffuser systems in one of the combinations listed in paragraph (b) (1), (2), (3), or (5) of this section or as a single agent listed in paragraph (b) (4) or (6) of this section. Quantities of the individual additives in parts per million are expressed in terms of the weight of the raw cane or raw beets.

(1) Combination for cane-sugar mills:

	Parts per million
Disodium cyanodithioimidocarbonate.....	2.5
Ethylenediamine.....	1.0
Potassium N-methyldithiocarbamate.....	3.5

(2) Combination for cane-sugar mills:

	Parts per million

Disodium ethylenebisdithiocarbamate	3.0
Sodium dimethyldithiocarbamate	3.0

(3) Combinations for cane-sugar mills and beet-sugar mills:

	Parts per million
(i) Disodium ethylenebisdithiocarbamate	3.0
Ethylenediamine	2.0

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Sodium dimethyldithiocarbamate	3.0
(ii) Disodium cyanodithioimidocarbonate	2.9
Potassium N-methyldithiocarbamate	4.1

(4) Single additive for cane-sugar mills and beet-sugar mills.

	Parts per million
2,2-Dibromo-3-nitrilopropionamide (CAS Reg. No. 10222-01-2). Limitations: Byproduct molasses, bagasse, and pulp containing residues of 2,2-dibromo-3-nitrilopropionamide are not authorized for use in animal feed..	Not more than 10.0 and not less than 2.0.

(5) Combination for cane-sugar mills:

	Parts per million
n-Dodecyl dimethyl benzyl ammonium chloride	0.05<SUP>plus-minus 0.005
n-Dodecyl dimethyl ethylbenzyl ammonium chloride	0.68<SUP>plus-minus 0.068
n-Hexadecyl dimethyl benzyl ammonium chloride	0.30<SUP>plus-minus 0.030
n-Octadecyl dimethyl benzyl ammonium chloride	0.05<SUP>plus-minus 0.005
n-Tetradecyl dimethyl benzyl ammonium chloride	0.60<SUP>plus-minus 0.060
n-Tetradecyl dimethyl ethylbenzyl ammonium chloride	0.32<SUP>plus-minus 0.032

Limitations. Byproduct molasses, bagasse, and pulp containing residues of these quaternary ammonium salts are not authorized for use in animal feed.

(6) Single additive for beet-sugar mills:

	Parts per million
Glutaraldehyde (CAS Reg. No. 111-30-8).	Not more than 250.

(c) To assure safe use of the additives, their label and labeling shall conform to that registered with the Environmental Protection Agency.

[42 FR 14526, Mar. 15, 1977, as amended at 47 FR 35756, Aug. 17, 1982; 50 FR 3891, Jan. 29, 1985; 57 FR 8065, Mar. 6, 1992]

Sec. 173.322 Chemicals used in delinting cottonseed.

Chemicals may be safely used to assist in the delinting of cottonseed in accordance with the following conditions:

(a) The chemicals consist of one or more of the following:

(1) Substances generally recognized as safe for direct addition to food.

(2) Substances identified in this paragraph and subject to such limitations as are provided:

Substances	Limitations
alpha-Alkyl-omega-hydroxypoly-(oxyethylene) produced by condensation of a linear primary alcohol containing an average chain length of 10 carbons with poly(oxyethylene) having an average of 5 ethylene oxide units.	May be used at an application rate not to exceed 0.3 percent by weight of cottonseeds to enhance delinting of cottonseeds intended for the production of cottonseed oil. Byproducts including lint, hulls, and meal may be used in animal feed.
An alkanomide produced by condensation of coconut oil fatty acids and diethanolamine, CAS Reg. No. 068603-42-9.	May be used at an application rate not to exceed 0.2 percent by weight of cottonseeds to enhance delinting of cottonseeds intended for the production of cottonseed oil. Byproducts including lint, hulls, and meal may be used in animal feed.

[47 FR 8346, Feb. 26, 1982]

Sec. 173.325 Acidified sodium chlorite solutions.

Acidified sodium chlorite solutions may be safely used in accordance with the following prescribed conditions:

(a) The additive is produced by mixing an aqueous solution of sodium chlorite (CAS Reg. No. 7758-19-2) with any generally recognized as safe (GRAS) acid.

(b) The additive is used as an antimicrobial agent in poultry processing water as a component of a carcass spray or dip solution prior to immersion of the carcass in a prechiller or chiller tank, or in a prechiller or chiller solution in accordance with current industry practice for use of poultry process water.

(1) When used in a carcass spray or dip solution, the additive is used at levels that result in sodium chlorite concentrations between 500 and 1,200 parts per million (ppm), in combination with any GRAS acid at levels sufficient to achieve a solution pH of 2.5 to 2.9.

(2) When used in a prechiller or chiller tank, the additive is used at levels that result in sodium chlorite concentrations between 50 and 150 ppm, in combination with any GRAS acid at levels sufficient to achieve a solution pH of 2.8 to 3.2.

(c) The additive is used as an antimicrobial agent in the processing of

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red meat as a component of a carcass spray in accordance with current industry practice. In the carcass spray, the additive is used at levels that result in sodium chlorite concentrations between 500 and 1,200 parts per million (ppm) in combination with any GRAS acid at levels sufficient to achieve a solution pH of 2.5 to 2.9.

(d) The concentration of sodium chlorite is determined by a method entitled ``Determination of Sodium Chlorite: 50 ppm to 1500 ppm Concentration,'' September 13, 1995, developed by Alcide Corp., Redmond, WA, which is incorporated by reference in accordance with 5 U.S.C. 552(a) and 1 CFR part 51. Copies are available from the Division of Petition Control (HFS-215), Center for Food Safety and Applied Nutrition, Food and Drug Administration, 200 C St. SW., Washington, DC 20204-0001, or may be examined at the Center for Food Safety and Applied Nutrition's Library, 200 C St. SW., rm. 3321, Washington, DC 20204-0001, or the Office of the Federal Register, 800 North Capitol St. NW., suite 700, Washington, DC.

[61 FR 17829, Apr. 23, 1996, as amended at 63 FR 11119, Mar. 6, 1998]

Sec. 173.340 Defoaming agents.

Defoaming agents may be safely used in processing foods, in accordance with the following conditions:

(a) They consist of one or more of the following:

(1) Substances generally recognized by qualified experts as safe in food or covered by prior sanctions for the use prescribed by this section.

(2) Substances listed in this paragraph (a)(2) of this section, subject to any limitations imposed:

Substances	Limitations
Dimethylpolysiloxane (substantially free from hydrolyzable chloride and alkoxy groups; no more than 18 percent loss in weight after heating 4 hours at 200 deg.C; viscosity 300 to 1,050 centistokes at 25 deg.C; refractive index 1.400-1.404 at 25 deg.C).	10 parts per million in food, or at such level in a concentrated food that when prepared as directed on the labels, the food in its ready-for-consumption state will have not more than 10 parts per million except as follows: Zero in milk; 110 parts per million in dry gelatin dessert mixes labeled for use whereby no more than 16 parts

	per million is present in the ready-to-serve dessert; 250 parts per million in salt labeled for cooking purposes, whereby no more than 10 parts per million is present in the cooked food.
Formaldehyde	As a preservative in defoaming agents containing dimethylpolysiloxane, in an amount not exceeding 1.0 percent of the dimethylpolysiloxane content.
<greek-a>-Hydro-omega-hydroxy-poly (oxyethylene)/poly(oxypropylene) (minimum 15 moles)/ poly(oxyethylene) block copolymer (CAS Reg. No. 9003-11-6) as defined in Sec. 172.808(a)(3) of this chapter.	For use as prescribed in Sec. 172.808(b)(3) of this chapter.
Polyacrylic acid, sodium salt	As a stabilizer and thickener in defoaming agents containing dimethylpolysiloxane in an amount reasonably required to accomplish the intended effect.
Polyethylene glycol	As defined in Sec. 172.820 of this chapter.
Polyoxyethylene 40 monostearate	As defined in U.S.P. XVI.
Polysorbate 60	As defined in Sec. 172.836 of this chapter.
Polysorbate 65	As defined in Sec. 172.838 of this chapter.
Propylene glycol alginate	As defined in Sec. 172.858 of this chapter.
Silicon dioxide	As defined in Sec. 172.480 of this chapter.
Sorbitan monostearate	As defined in Sec. 172.842 of this chapter.
White mineral oil: Conforming with Sec. 172.878 of this chapter.	As a component of defoaming agents for use in wash water for sliced potatoes at a level not to exceed 0.008 percent of the wash water.

Substances	Limitations
Aluminum stearate	As defined in Sec. 172.863 of this chapter.
Butyl stearate	
BHA	As an antioxidant, not to exceed 0.1 percent by weight of defoamer.
BHT	Do.
Calcium stearate	As defined in Sec. 172.863 of this chapter.
Fatty acids	As defined in Sec. 172.860 of this chapter.

Formaldehyde.....	As a preservative.
Hydroxylated lecithin.....	As defined in Sec. 172.814 of this chapter.
Isopropyl alcohol.....	
Magnesium stearate.....	As defined in Sec. 172.863 of this chapter.
Mineral oil: Conforming with Sec. 172.878 of this chapter.	Not more than 150 p.p.m. in yeast, measured as hydrocarbons.
Odorless light petroleum hydrocarbons: Conforming with Sec. 172.884 of this chapter.	.....
Petrolatum: Conforming with Sec. 172.880 of this chapter.	.....
Petroleum wax: Conforming with Sec. 172.886 of this chapter.	
Petroleum wax, synthetic.....	
Polyethylene glycol (400)dioleate: Conforming with Sec. 172.820(a)(2) of this chapter and providing the oleic acid used in the production of this substance complies with Sec. 172.860 or Sec. 172.862 of this chapter.	As an emulsifier not to exceed 10 percent by weight of defoamer formulation.
Synthetic isoparaffinic petroleum hydrocarbons: Conforming with Sec. 172.882 of this chapter.	
Oleic acid derived from tall oil fatty acids.	Complying with Sec. 172.862 of this chapter.
Oxystearin.....	As defined in Sec. 172.818 of this chapter.
Polyoxyethylene (600) dioleate.....	
Polyoxyethylene (600) monoricinoleate..	
Polypropylene glycol.....	Molecular weight range, 1,200-3,000.
Polysorbate 80.....	As defined in Sec. 172.840 of this chapter.
Potassium stearate.....	As defined in Sec. 172.863 of this chapter.
Propylene glycol mono- and diesters of fats and fatty acids.	As defined in Sec. 172.856 of this chapter.
Soybean oil fatty acids, hydroxylated..	
Tallow, hydrogenated, oxidized or sulfated.	
Tallow alcohol, hydrogenated.....	

(4) The substance listed in this paragraph (a)(4), provided it is a component of defoaming agents limited to use in processing beet sugar only, and subject to the limitations imposed:

Substance	Limitations
n-Butoxypoly(oxyethylene)-poly(oxypropylene)glycol.	Viscosity range, 4,850-5,350 Saybolt Universal Seconds (SUS) at 37.8 deg.C (100 deg.F). The viscosity range is determined by the method ``Viscosity Determination

of n-butoxypoly(oxyethylene)-poly(oxypropylene) glycol'' dated April 26, 1995, developed by Union Carbide Corp., P.O. Box 670, Bound Brook, NJ 08805, which is incorporated by reference in accordance with 5 U.S.C. 552(a) and 1 CFR part 51. Copies of the material incorporated by reference are available from the Division of Petition Control, Center for Food Safety and Applied Nutrition (HFS-215), Food and Drug Administration, 200 C St. SW., Washington, DC 20204, and may be examined at the Center for Food Safety and Applied Nutrition's Library, 200 C St. SW., rm. 3321, Washington, DC, or at the Office of the Federal Register, 800 North Capitol St. NW., suite 700, Washington, DC.

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(b) They are added in an amount not in excess of that reasonably required to inhibit foaming.
[42 FR 14526, Mar. 15, 1977, as amended at 43 FR 2872, Jan. 20, 1978; 46 FR 30493, June 9, 1981; 46 FR 57476, Nov. 24, 1981; 60 FR 54036, Oct. 19, 1995; 61 FR 632, Jan. 9, 1996]

Sec. 173.342 Chlorofluorocarbon 113 and perfluorohexane.

A mixture of 99 percent chlorofluorocarbon 113 (1,1,2-trichloro-1,2,2-trifluoroethane) (CAS Reg. No. 76-13-1, also known as fluorocarbon 113, CFC 113 and FC 113) and 1 percent perfluorohexane (CAS Reg. No. 355-42-0) may be safely used in accordance with the following prescribed conditions:

(a) The additive chlorofluorocarbon 113 has a purity of not less than 99.99 percent.

(b) The additive mixture is intended for use to quickly cool or crust-freeze chickens sealed in intact bags composed of substances regulated in parts 174, 175, 177, 178, and Sec. 179.45 of this chapter and conforming to any limitations or specifications in such regulations.
[55 FR 8913, Mar. 9, 1990]

Sec. 173.345 Chloropentafluoroethane.

The food additive chloropentafluoroethane may be safely used in food in accordance with the following prescribed conditions:

(a) The food additive has a purity of not less than 99.97 percent, and contains not more than 200 parts per million saturated fluoro compounds and 10 parts per million unsaturated fluoro compounds as impurities.

(b) The additive is used or intended for use alone or with one or more of the following substances: Carbon dioxide, nitrous oxide, propane, and octafluorocyclobutane complying with Sec. 173.360, as an aerating agent for foamed or sprayed food products, with any propellant effect being incidental and no more than is minimally necessary to achieve the aerating function, except that use is not permitted for those standardized foods that do not provide for such use.

(c) To assure safe use of the additive

(1) The label of the food additive container shall bear, in addition to the other information required by the act, the following:

(i) The name of the additive, chloropentafluoroethane.

(ii) The percentage of the additive present in the case of a mixture.

(iii) The designation ``food grade''.

(2) The label or labeling of the food additive container shall bear adequate directions for use.

[42 FR 14526, Mar. 15, 1977, as amended at 43 FR 11317, Mar. 17, 1978; 43 FR 14644, Apr. 7, 1978]

Sec. 173.350 Combustion product gas.

The food additive combustion product gas may be safely used in the processing and packaging of the foods designated in paragraph (c) of this section for the purpose of removing and displacing oxygen in accordance with the following prescribed conditions:

(a) The food additive is manufactured by the controlled combustion in air of butane, propane, or natural gas. The combustion equipment shall be provided with an absorption-type filter capable of removing possible toxic impurities, through which all gas used in the treatment of food shall pass; and with suitable controls to insure that any combustion products failing to meet the specifications provided in this section will be prevented from reaching the food being treated.

(b) The food additive meets the following specifications:

(1) Carbon monoxide content not to exceed 4.5 percent by volume.

(2) The ultraviolet absorbance in isooctane solution in the range 255 millimicrons to 310 millimicrons not to exceed one-third of the standard reference absorbance when tested as described in paragraph (e) of this section.

(c) It is used or intended for use to displace or remove oxygen in the processing, storage, or packaging of beverage products and other food, except fresh meats.

(d) To assure safe use of the additive in addition to the other information required by the act, the label or labeling of the combustion device shall bear adequate directions for use to provide a combustion product gas that complies with the limitations prescribed in paragraph (b) of this section, including instructions to assure proper filtration.

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(e) The food additive is tested for compliance with paragraph (b)(2) by the following empirical method:

Spectrophotometric measurements. All measurements are made in an ultraviolet spectrophotometer in optical cells of 5 centimeters in length, and in the range of 255 millimicrons to 310 millimicrons, under the same instrumental conditions. The standard reference absorbance is

the absorbance at 275 millimicrons of a standard reference solution of naphthalene (National Bureau of Standards Material No. 577 or equivalent in purity) containing a concentration of 1.4 milligrams per liter in purified isooctane, measured against isooctane of the same spectral purity in 5-centimeter cells. (This absorbance will be approximately 0.30.)

Solvent. The solvent used is pure grade isooctane having an ultraviolet absorbance not to exceed 0.05 measured against distilled water as a reference. Upon passage of purified inert gas through some isooctane under the identical conditions of the test, a lowering of the absorbance value has been observed. The absorbance of isooctane to be used in this procedure shall not be more than 0.02 lower in the range 255 millimicrons to 310 millimicrons, inclusive, than that of the untreated solvent as measured in a 5-centimeter cell. If necessary to obtain the prescribed purities, the isooctane may be passed through activated silica gel.

Apparatus. To assure reproducible results, the additive is passed into the isooctane solution through a gas-absorption train consisting of the following components and necessary connections:

1. A gas flow meter with a range up to 30 liters per hour provided with a constant differential relay or other device to maintain a constant flow rate independent of the input pressure.

2. An absorption apparatus consisting of an inlet gas dispersion tube inserted to the bottom of a covered cylindrical vessel with a suitable outlet on the vessel for effluent gas. The dimensions and arrangement of tube and vessel are such that the inlet tube introduces the gas at a point not above 5\1/4\ inches below the surface of the solvent through a sintered glass outlet. The dimensions of the vessel are such, and both inlet and vessel are so designed, that the gas can be bubbled through 60 milliliters of isooctane solvent at a rate up to 30 liters per hour without mechanical loss of solvent. The level corresponding to 60 milliliters should be marked on the vessel.

3. A cooling bath containing crushed ice and water to permit immersion of the absorption vessel at least to the solvent level mark.

Caution. The various parts of the absorption train must be connected by gas-tight tubing and joints composed of materials which will neither remove components from nor add components to the gas stream. The gas source is connected in series to the flow-rate device, the flow meter, and the absorption apparatus in that order. Ventilation should be provided for the effluent gases which may contain carbon monoxide.

Sampling procedure. Immerse the gas-absorption apparatus containing 60 milliliters of isooctane in the coolant bath so that the solvent is completely immersed. Cool for at least 15 minutes and then pass 120 liters of the test gas through the absorption train at a rate of 30 liters per hour or less. Maintain the coolant bath at 0 deg.C throughout. Remove the absorption vessel from the bath, disconnect, and warm to room temperature. Add isooctane to bring the contents of the absorption vessel to 60 milliliters, and mix. Determine the absorbance of the solution in the 5-centimeter cell in the range 255 millimicrons to 310 millimicrons, inclusive, compared to isooctane. The absorbance of the solution of combustion product gas shall not exceed that of the isooctane solvent at any wavelength in the specified range by more than one-third of the standard reference absorbance.

Sec. 173.355 Dichlorodifluoromethane.

The food additive dichlorodifluoromethane may be safely used in food in accordance with the following prescribed conditions:

(a) The additive has a purity of not less than 99.97 percent.

(b) It is used or intended for use, in accordance with good

manufacturing practice, as a direct-contact freezing agent for foods.

(c) To assure safe use of the additive:

(1) The label of its container shall bear, in addition to the other information required by the act, the following:

(i) The name of the additive, dichlorodifluoromethane, with or without the parenthetical name ``Food Freezant 12''.

(ii) The designation ``food grade''.

(2) The label or labeling of the food additive container shall bear adequate directions for use.

Sec. 173.357 Materials used as fixing agents in the immobilization of enzyme preparations.

Fixing agents may be safely used in the immobilization of enzyme preparations in accordance with the following conditions:

(a) The materials consist of one or more of the following:

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(1) Substances generally recognized as safe in food.

(2) Substances identified in this subparagraph and subject to such limitations as are provided:

Substances	Limitations
Cellulose triacetate.....	May be used as a fixing material in the immobilization of lactase for use in reducing the lactose content of milk.
Diethylaminoethyl-cellulose.....	May be used as a fixing material in the immobilization of glucose isomerase enzyme preparations for use in the manufacture of high fructose corn syrup, in accordance with Sec. 184.1372 of this chapter.
Glutaraldehyde.....	Do.
Periodic acid (CAS Reg. No. 10450-60-9)..	
Polyethylenimine reaction product with 1,2-dichloroethane (CAS Reg.No. 68130-97-2) is the reaction product of homopolymerization of ethylenimine in aqueous hydrochloric acid at 100 deg.C and of cross-linking with 1,2-dichloroethane. The finished polymer has an average molecular weight of 50,000 to 70,000 as determined by gel permeation chromatography. The analytical method is entitled ``Methodology for Molecular Weight Detection of Polyethylenimine,'' which is incorporated by reference in accordance with 5 U.S.C. 552(a) and 1 CFR part 51. Copies may be obtained from the Division of Petition Control, Center for Food Safety and Applied Nutrition (HFS-	May be used as a fixing material in the immobilization of glucoamylase enzyme preparations from Aspergillus niger for use in the manufacture of beer. May be used as a fixing material in the immobilization of: 1. Glucose isomerase enzyme preparations for use in the manufacture of high fructose corn syrup, in accordance with Sec. 184.1372 of this chapter. 2. Glucoamylase enzyme preparations from Aspergillus niger for use in the manufacture of beer. Residual ethylenimine in the finished polyethylenimine polymer will be less than 1 part per million as determined by gas chromatography-mass

200), 200 C St. SW., Washington, DC 20204, and may be examined at the Center for Food Safety and Applied Nutrition's Library, 200 C St. SW., rm. 3321, Washington, DC, or at the Office of the Federal Register, 800 North Capitol St. NW., suite 700, Washington, DC..

spectrometry. The residual ethylenimine is determined by an analytical method entitled ``Methodology for Ethylenimine Detection in Polyethylenimine,'' which is incorporated by reference in accordance with 5 U.S.C. 552(a) and 1 CFR part 51. Residual 1,2-dichloroethane in the finished polyethylenimine polymer will be less than 1 part per million as determined by gas chromatography. The residual 1,2-dichloroethane is determined by an analytical method entitled, ``Methodology for Ethylenedichloride Detection in Polyethylenimine,'' which is incorporated by reference in accordance with 5 U.S.C. 552(a) and 1 CFR part 51. Copies may be obtained from the Division of Petition Control, Center for Food Safety and Applied Nutrition (HFS-215), 200 C St. SW., Washington, DC 20204, or may be examined at the Center for Food Safety and Applied Nutrition's Library, 200 C St. SW., rm. 3321, Washington, DC, or the Office of the Federal Register, 800 North Capitol St. NW., suite 700, Washington, DC.

(b) The fixed enzyme preparation is washed to remove residues of the fixing materials.

[48 FR 5716, Feb. 8, 1983, as amended at 52 FR 39512, Oct. 22, 1987; 55 FR 12172, Apr. 2, 1990; 59 FR 36937, July 20, 1994; 61 FR 4873, Feb. 9, 1996; 61 FR 14245, Apr. 1, 1996]

Sec. 173.360 Octafluorocyclobutane.

The food additive octafluorocyclo-butane may be safely used as a propellant and aerating agent in foamed or sprayed food products in accordance with the following conditions:

(a) The food additive meets the following specifications:

99.99 percent octafluorocyclobutane.
Less than 0.1 part per million fluoroolefins, calculated as perfluoroisobutylene.

(b) The additive is used or intended for use alone or with one or more of the following substances: Carbon dioxide, nitrous oxide, and propane, as a propellant and aerating agent for foamed or sprayed food products, except for those standardized foods that do not provide for such use.

(c) To assure safe use of the additive:

(1) The label of the food additive container shall bear, in addition to the other information required by the act, the following:

(i) The name of the additive, octafluorocyclobutane.
(ii) The percentage of the additive present in the case of a mixture.
(iii) The designation ``food grade''.
(2) The label or labeling of the food additive container shall bear adequate directions for use.

Sec. 173.385 Sodium methyl sulfate.

Sodium methyl sulfate may be present in pectin in accordance with the following conditions.
(a) It is present as the result of methylation of pectin by sulfuric acid and methyl alcohol and subsequent treatment with sodium bicarbonate.

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(b) It does not exceed 0.1 percent by weight of the pectin.

Sec. 173.395 Trifluoromethane sulfonic acid.

Trifluoromethane sulfonic acid has the empirical formula CF!<INF>3</INF> SO!<INF>3</INF> H (CAS Reg. No. 1493-13-6). The catalyst (Trifluoromethane sulfonic acid) may safely be used in the production of cocoa butter substitute from palm oil (1-palmitoyl-2-oleoyl-3-stearin) (see Sec. 184.1259 of this chapter) in accordance with the following conditions:
(a) The catalyst meets the following specifications:

Appearance, Clear liquid.
Color, Colorless to amber.
Neutralization equivalent, 147-151.
Water, 1 percent maximum.
Fluoride ion, 0.03 percent maximum.
Heavy metals (as Pb), 30 parts per million maximum.
Arsenic (as As), 3 parts per million maximum.

(b) It is used at levels not to exceed 0.2 percent of the reaction mixture to catalyze the directed esterification.
(c) The esterification reaction is quenched with steam and water and the catalyst is removed with the aqueous phase. Final traces of catalyst are removed by washing batches of the product three times with an aqueous solution of 0.5 percent sodium bicarbonate.
(d) No residual catalyst may remain in the product at a detection limit of 0.2 part per million fluoride as determined by the method described in ``Official Methods of Analysis of the Association of Official Analytical Chemists,'' sections 25.049-25.055, 13th Ed. (1980), which is incorporated by reference. Copies may be obtained from the Association of Official Analytical Chemists International, 481 North Frederick Ave., suite 500, Gaithersburg, MD 20877-2504, or may be examined at the Office of the Federal Register, 800 North Capitol Street, NW., suite 700, Washington, DC 20408.
[43 FR 54237, Nov. 11, 1978, as amended at 49 FR 10106, Mar. 19, 1984; 54 FR 24897, June 12, 1989]

Sec. 173.400 Dimethyldialkylammonium chloride.

Dimethyldialkylammonium chloride may be safely used in food in accordance with the following prescribed conditions:
(a) The food additive is produced by one of the following methods:

(1) Ammonolysis of natural tallow fatty acids to form amines that are subsequently reacted with methyl chloride to form the quaternary ammonium compounds consisting primarily of dimethyldioctadecylammonium chloride and dimethyldihexadecylammonium chloride. The additive may contain residues of isopropyl alcohol not in excess of 18 percent by weight when used as a processing solvent.

(2) Ammonolysis of natural tallow fatty acids to form amines that are then reacted with 2-ethylhexanal, reduced, methylated, and subsequently reacted with methyl chloride to form the quaternary ammonium compound known as dimethyl(2-ethylhexyl) hydrogenated tallow ammonium chloride and consisting primarily of dimethyl(2-ethylhexyl)octadecylammonium chloride and dimethyl(2-ethylhexyl)hexadecylammonium chloride.

(b) The food additive described in paragraph (a)(1) of this section contains not more than a total of 2 percent by weight of free amine and amine hydrochloride. The food additive described in paragraph (a)(2) of this section contains not more than 3 percent by weight, each, of free amine and amine hydrochloride as determined by A.O.C.S. method Te 3a-64, ``Acid Value and Free Amine Value of Fatty Quaternary Ammonium Chlorides,'' 2d printing including additions and revisions 1990, which is incorporated by reference in accordance with 5 U.S.C. 552(a) and 1 CFR part 51. Copies are available from the Center for Food Safety and Applied Nutrition (HFS-200), Food and Drug Administration, 200 C St. SW., Washington, DC 20204, and from the American Oil Chemists' Society, P.O. Box 5037, Station A, Champaign, IL 61820, or available for inspection at the Office of the Federal Register, 800 North Capitol Street, NW., suite 700, Washington, DC.

(c) The food additive is used as a decolorizing agent in the clarification of

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refinery sugar liquors under the following limitations:

(1) The food additive described in paragraph (a)(1) of this section is added only at the defecation/clarification stage of sugar liquor refining in an amount not to exceed 700 parts per million by weight of sugar solids.

(2) The food additive described in paragraph (a)(2) of this section is used under the following conditions:

(i) The additive is adsorbed onto a support column composed of suitable polymers that are regulated for contact with aqueous food. Excess nonadsorbed additive shall be rinsed away with potable water prior to passage of sugar liquor through the column.

(ii) The residue of the additive in the decolorized sugar liquor prior to crystallization shall not exceed 1 part per million of sugar as determined by a method entitled ``Colorimetric Determination of Residual Quaternary Ammonium Compounds (Arquad HTL8) in Sugar and Sugar Solutions,'' June 13, 1990, which is incorporated by reference in accordance with 5 U.S.C. 552(a) and 1 CFR part 51. Copies are available from the Center for Food Safety and Applied Nutrition (HFS-200), Food and Drug Administration, 200 C St. SW., Washington, DC 20204, or available for inspection at the Office of the Federal Register, 800 North Capitol Street, NW., suite 700, Washington, DC.

(d) To assure safe use of the additive, the label and labeling of the additive shall bear, in addition to other information required by the Federal Food, Drug, and Cosmetic Act, adequate directions to assure use in compliance with paragraph (c) of this section.

[56 FR 42686, Aug. 29, 1991]

6.3.1

6.3.H Clarifying agent in beverages and vinegar: PVP

See following pages from Code of Federal Regulations.

[Code of Federal Regulations]
[Title 21, Volume 3, Parts 170 to 199]
[Revised as of April 1, 1998]
From the U.S. Government Printing Office via GPO Access
[CITE: 21CFR173.55]

[Page 116]

TITLE 21--FOOD AND DRUGS

CHAPTER I--FOOD AND DRUG ADMINISTRATION, DEPARTMENT OF HEALTH AND HUMAN SERVICES (

PART 173--SECONDARY DIRECT FOOD ADDITIVES PERMITTED IN FOOD FOR HUMAN CONSUMPTION-

Subpart A--Polymer Substances and Polymer Adjuvants for Food Treatment

Sec. 173.55 Polyvinylpyrrolidone.

The food additive polyvinylpyrroli-done may be safely used in accordance with the following prescribed conditions:

(a) The additive is a polymer of purified vinylpyrrolidone catalytically produced, having an average molecular weight of 40,000 and a maximum unsaturation of 1 percent, calculated as the monomer, except that the polyvinylpyrrolidone used in beer is that having an average molecular weight of 360,000 and a maximum unsaturation of 1 percent, calculated as the monomer.

(b) The additive is used or intended for use in foods as follows:

Food	Limitations
Beer	As a clarifying agent, at a residual level not to exceed 10 parts per million.
Flavor concentrates in tablet form.	As a tableting adjuvent in an amount not to exceed good manufacturing practice.
Nonnutritive sweeteners in concentrated liquid form.	As a stabilizer, bodying agent, and dispersant, in an amount not to exceed good manufacturing practice.
Nonnutritive sweeteners in tablet form.	As a tableting adjuvant in an amount not to exceed good manufacturing practice.
Vitamin and mineral concentrates in liquid form.	As a stabilizer, bodying agent, and dispersant, in an amount not to exceed good manufacturing practice.
Vitamin and mineral concentrates in tablet form.	As a tableting adjuvant in an amount not to exceed good manufacturing practice.
Vinegar	As a clarifying agent, at a residual level not to exceed 40 parts per million.
Wine	As a clarifying agent, at a residual level not to exceed 60 parts per million.

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SUBMISSION FILE

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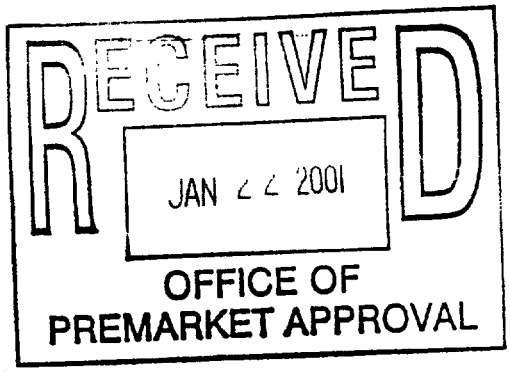
December 18, 2000



Office of Premarket Approval: **GRAS Exemption Claim**
Food and Drug Administration
Center for Food Safety and Applied Nutrition
Department of Health and Human Services
Washington, D.C. 20204

Dear Madam or Sir

Pursuant to the proposed 21C.F.R. §170.35 (c) (1), ShanStar Biotech, Incorporated hereby claims that ShanStar Cranberry extract produced by adsorption of cranberry bioflavonoids to USP Grade Crospovidone, is Generally Recognized As Safe (GRAS).
For this reason, it is exempt from statutory premarket approval.

In accordance with the proposed regulation, the following information is provided:

1) The name and address of the notifier

ShanStar Biotech, Incorporated
P.O. Box 10
Chadwick Bay, New York 14048

2) The common or usual name of the notified substance

Cranberry-Crospovidone extract, prepared from cranberry juice and Crospovidone NF.

3) Applicable conditions of use

ShanStar Cranberry extract is to be used in a limited market of the food and beverage industry, primarily to fortify foods and beverages with bioflavonoids that have been shown to support maintenance of a healthy urinary tract and cardiovascular system.

The two markets where ShanStar Cranberry will be used are:

- Fortification of desserts served to patients in long term nursing facilities and
- Fortification of beverages served to patients in long term nursing facilities.

The objective is to provide these persons with minimal amounts of required phytonutrients because they are unable to eat or drink the recommended number of daily servings of fruits and vegetables.

4) Basis for GRAS determination

The GRAS determination is based on scientific procedure, published ADI values for Povidone, and published reports on the toxicity of Crospovidone. In addition to our notification, Dr James Griffiths of ISP has informed me that they are applying for a broad scope GRAS for

000380

Box 10 Chadwick Bay NY 14048

Crospovidone in foods. They plan to gather an expert committee that will include Dr Joseph Borzellaca, a recognized expert on PVP and PVPP.

5) Availability of information.

Three copies of our notification package are enclosed both as hard copies and electronically as CD-ROM's. The software platform used is Acrobat Reader 4.05 (also supplied on the CD). The electronic format also makes it much easier for you to review and share the information within the agency.

The package includes an oral toxicity study, an evaluation of dietary exposure to the limited market group, quantifications of quercetin, myricetin and kaempferol in the product, the manufacturing process, in vitro microbial inhibition study, HPLC analysis and comparisons to pure cranberry juice, and various other data. Also included is a complete listing of published literature on clinical studies on cranberry juice and powder, and studies of Povidone and Crospovidone. Complete data and information that are the basis of this GRAS determination are available to the Food and Drug Administration for review and co[illegible] request.

Crospovidone (PVPP) has a drug master file, type V (#2297) on file [illegible] P, the manufacturer of Crospovidone NF has told us that much of the relevant c[illegible] cluded in this file. At this time they have refused to give a copy of the file to ShanS[illegible] proprietary nature. However, they have assured me that the data in the file woul[illegible] ive to this application. You, as the FDA, however, should have ready access to this DMF. [illegible] se consider this data in your review of this file.

To the best of my knowledge, the information contained herein represents a balanced submission that includes unfavorable as well as favorable information, known to be pertinent to the evaluation of the safety and functionality of ShanStar Cranberry extract.

Sincerely yours,

Date:

12/18/2000

David Marciniak
Manager of Regulatory Affairs,
Quality and Customer Product Services
Cliffstar Corporation*

* Cliffstar Corporation, of 1 Cliffstar Avenue, Dunkirk, NY 14048, wholly owns and operates ShanStar Biotech, Incorporated

[redacted]

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[redacted]

[redacted]

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Section 1 Product Description

1.1 Name of Substance

1.2 Food Grade Specification

1.3 Quantitative Composition and Methods

1.4 Summary of Manufacturing Process with Schematic

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[redacted]

Section 1.1 Name of Substance

The common or usual name of this substance is crospovidone-cranberry extract.

The official product name for this substance is ShanStar™ Cranberry extract.

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Section 1.2 Food Grade Specification

1.2.A Description

1.2.A.1 Product Definition

ShanStar Cranberry is an extract of cranberry containing bioflavonoids and/or polyphenolics found in cranberry fruit, and the USP-NF grade disintegrant, Crospovidone.

(a) ShanStar Cranberry extract is prepared from the juice of washed, frozen, cranberries and USP grade disintegrant, Crospovidone- NF.

(b) The cranberries used in the preparation of the juice conform to the characteristics of the fruit of *Vaccinium macrocarpon.*

(c) The Crospovidone conforms to the characteristics outlined in the Food Chemical Codex, Fourth Edition, National Academy Press, Washington, D.C., Effective July 1, 1996.

(d) The Crospovidone-cranberry extract is made according to the process outlined in Section 1.4.

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1.2.A.2 Product style

The product is a solid consisting of cranberry extract adsorbed to Crospovidone. Crospovidone is not more than 60% of the total mass, water not more than 10% and cranberry extract not less than 30%.

1.2.A.3 Product Specification

Specifications as listed on a Certificate of Analysis for ShanStar Cranberry extract

ShanStar Cranberry extract — Lot No.: D990423

ANALYSIS	RESULTS	SPECIFICATIONS
Description		
APPEARANCE:	Conforms	Cranberry Red
FLAVOR:	Conforms	Bitter
Physical/Chemical		
TOTAL PHENOLICS (GAE/g):	Conforms	220 +/- 50 mg
pH (5% in water)	Conforms	2.4 – 3.0
MOISTURE	Conforms	<10%
Biological		
TOTAL PLATE COUNT	Conforms	<1000 cfu/g
YEAST	Conforms	<100 cfu/g
MOLDS	Conforms	<100 cfu/g
COLIFORMS	Not Present	<10 cfu/g
SALMONELLA (per 30g)	Not Present	Negative
E. COLI (per 10g)	Not Present	Negative

[redacted]

1.2.A.4 Classification of defectives

A batch of product that fails to meet the specifications, as given in Section A.3 above, shall be considered defective.

1.2.A.5 Acceptance

A lot will be considered accepted when all QC tests are within the limits of the specifications as given in Section A.3.

1.2.B Composition and Quality Factors

1.2.B.1 Ingredients

1.2.B.1.1 Approximately 30% of ShanStar Cranberry consist of components of cranberry fruit that adsorb to USP-NF Crospovidone. Of this 30%, ~ 50% are polyphenolics such as anthocyanins, proanthocyanidins and a variety of other natural bioflavonoids found in cranberries. See the chromatographic profiles at 520 nm in Section 4.2.

1.2.B.1.2 Approximately 10% of ShanStar Cranberry consists of water.

1.2.B.1.3 Not more than 60% of the weight of ShanStar Cranberry consists of USP-NF Crospovidone.

1.2.B.1.4 ShanStar Cranberry contains trace amounts of natural acids and sugars (<1%) from cranberry fruit.

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[redacted]

1.2.B.2 Quality Criteria

1.2.B.2.1 Color

The product has a dark red to magenta color originating from the skins of cranberry.

1.2.B.2.2 Flavor

ShanStar Cranberry extract has a bitter, astringent taste in pure form. In foods, there is no flavor or taste contribution at levels up to several grams per 8 oz of juice or 3 oz of solids.

1.2.B.2.3 Consistency

The product consists of Crospovidone- NF, Cranberry extract and water. The product's consistency is similar to cornstarch.

1.2.B.2.4 Classification of "defectives"

Any batch that fails to meet any of the specifications as laid out in section 1.2.A.3 above shall be considered defective and shall be disposed of.

1.2.B.2.5 Acceptance

Product batches are accepted if they meet the specifications as laid out in section 1.2.A.3 above.

[redacted]

1.2.C Hygiene

1.2.C.1 The product is prepared in accordance with Good Manufacturing Practice and Hazard Analysis Critical Control Procedure (HACCP) is applied.

1.2.C.2 To the extent possible with Good Manufacturing Practice(21CFR Part 110, Subparts A-G, and HACCP) the product shall be free from objectionable matter.

1.2.C.3 When tested by appropriate methods of sampling and examination, the product:

(a) shall be free from micro-organisms capable of development under normal conditions of storage, and

(b) shall not contain any substances originating from microorganisms in amounts that may be hazardous to human or animal health.

1.2.D Weights and Measures

1.2.D.1Minimum fill

ShanStar Cranberry extract is available in containers as specified by the customer. Each package will be filled to a minimum of 101% of the specified weight.

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[redacted]

1.2.D.2Handling of defectives

If a package is delivered to a customer and it does not contain the minimum fill, additional material will be sent to the customer to make up the difference, or the package may be returned for a package with the correct fill.

1.2.E Labeling

The following information is provided on the product label. In addition, each package is accompanied by a Certificate of Analysis and the product's MSDS.

1.2.E.1 Name

The name of the product shall include the designation cranberry extract.

1.2.E.2 Name and Address of Manufacturer

The name and address of the manufacturer and vendor of the product is declared on the package.

1.2.E.3 Country of Origin

1.2.E.3.1 The country of origin shall be declared if its omission would mislead or deceive the consumer.

1.2.E.3.2 This product is to be wholly processed in the United States of America at ShanStar Biotech, Incorporated.

1.2.F Additional requirements

Since the product is to be used as an additive to foods and beverages for special dietary need, the table below will be added to the product label:

Use of ShanStar Cranberry	Type of Product	Maximum Amount to be Added
Special Dietary Need	Food	0.5 mg per gram of food
Special Dietary Need	Beverage	0.5 mg per ml of beverage
Regular Beverage	Beverage	0.1 mg per ml of beverage

This reminder is based on the already established, unspecified ADI for Crospovidone NF established by JECFA because it is considered totally inert and non-absorbed by the gastrointestinal tract, an Allowable Daily Intake (ADI) of 50 mg/kg of body weight for Povidone (PVP) as established by JECFA and the chemical fact that Crospovidone is more inert than Povidone.

In CFR 21, vol 3, part 175.105, "Additives used as adhesives for packaging in contact with food", it is stated that PVP and its related homo- and co-polymers, one of which is PVPP or Crospovidone, are considered equivalent.

Section 1.3 Quantitative Composition and Methods

1.3.A Composition

ShanStar Cranberry extract consists of the following components, per 1000 mg:

(b) (4)

1.3.B Methods

1.3.B.1 Crospovidone

The amount of Crospovidone per 1000 mg is determined by difference of the weight of sugars, acids, phenolics and water per gram.

1.3.B.2 Cranberry extract:

The quantification of cranberry extract consists of 2 different analyses, one for total phenolics and one for sugars and acids. The mg amounts of these substances are added together to arrive at the weight per gram of cranberry extract.

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1.3.B.2.1 Total phenolics

Total phenolics are determined using a modified AOAC Method 952.03 for total phenolics. The method has been modified by the use of Sigma's Folin Ciocalteu reagent instead of the Folin Denis reagent. The advantage is that the Folin Ciocalteu reagent is commercially available as a ready to use product unlike the Folin Denis reagent. The details of the method follow.

Solutions:

1) Folin-Ciocalteu reagent (Sigma Chemical)
2) 20% sodium carbonate solution
3) saturate sodium carbonate solution
4) 1 N hydrochloric acid
5) Gallic acid (Sigma Chemical)

Calibration:

1) Prepare gallic acid stock solution (0.25 mg/mL)
2) Transfer 1 mL gallic acid stock solution into a 200 mL volumetric flask. Fill to volume with deionized water.
3) Transfer 2 mL gallic acid stock solution into a 200 mL volumetric flask. Fill to volume with deionized water.
4) Into the two 100 mL flasks add:
   50 mL deionized water, 5 mL Folin-Ciocalteu, 15 mL 20% sodium carbonate solution
5) Let solutions stand for 2 hours and read absorbance are 760 nm.
6) Calculate the factors: $f=A_{760}/c$
   Where A760 is the absorbance at 760 nm and c is the gallic acid concentration (0.0025 mg/mL for calibration point 1 and 0.0050 mg/mL for calibration point 2).
7) Verify that the f values for the two points differ by less than 5%.
8) Repeat steps 2-8 two more times. Obtain response factor **F** by averaging the f values. Once an accurate response factor is established, it can be used for all future analysis. There is no need to perform the calibration every time a sample is submitted.

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Sample Preparation:

Due to the nature of the Shanstar product, the phenolics must be liberated before being analyzed. The extraction was accomplished by using 300 mg of Celite 560 loosely packed in a Bio-Rad Poly-Prep Chromatography Column and rinsed with 3 mL of 1 N HCl (to remove contaminants). The Celite was then mixed with 100 mg Shanstar and repacked into the column.

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To a 100 mL volumetric flask, add 50 mL 1 N HCl. Add a 15 mL aliquot of saturated sodium carbonate to the column and collect into the volumetric flask. Follow with 25 mL of 1 N HCl and 5 mL sat. sodium carbonate. Occasionally swirl the flask to help mix and

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liberate carbon dioxide. Shake or sonicate for five minutes. (Take caution! Release of CO_2 gas may be violent). Dilute to 100 mL with water.

To determine the phenolic content, 3 mL of the eluent was added to approximately 50 mL water; 5 mL Folin-Ciocalteu reagent and 15 mL 20% sodium carbonate were added and diluted to 100 mL with water.

Calculations:
%Polyphenols as GAE = $[(10000 \times A_{760}) / (c \times F \times (100-m)]$

A_{760}	=	Absorbance at 760 nm
c	=	concentration of sample (0.03 mg/mL for 3 mL sample)
F	=	factor determined above
m	=	percent moisture content

For milligrams phenolics as gallic acid per gram of sample, convert percent to mg/g.

1.3.B.2.2 Sugars and Acids

1) Activation of resin:
   a) Weigh approx. 1.1 g Bio-Rad Bio-Rex 5 Resin and place in Bio-Rad Poly-Prep Chromatography Column (or similar).
   b) Add 5 mL ~5 N NH_3OH and pack column as well as possible.
   c) Rinse with 2, 5 mL aliquots of water.
2) Sample:
   a) Weigh approx. 500 mg of dry sample.
   b) Add slowly to 5 mL water, try to prevent clumping, etc.
   c) Sonicate sample for 10 minutes to help dissolve sample (some products contain insoluble matter).
   d) Centrifuge for 10 min. at approx. 1200 rpm. (No. 9 setting on Fischer Scientific Centrifuge).
3) Separation of Sugars:
   a) Slowly add 2.5 mL supernatant to activated column and collect.
   b) Slowly add 5 mL water to rinse column.
   c) Collect rinse with previous elution.
4) Separation of Acids:
   a) Extract acids from resin using 2.5 mL 10 % H_2SO_4.
   b) Follow with 7.5 mL 0.01 N H_2SO_4.
   c) Collect eluent.

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Sugars:

HPLC method:

a) HPLC grade water for mobile phase.
b) ion-exchange column (Phenomenox 10 µm, 300 x 7.80 mm, 8% cross linked, calcium form used).
c) Temperature: 80 °C
d) RI detector
e) Flow rate: 0.6 mL/min.
f) Standards: 1.5 % sucrose (w/v), 2.5 % glucose (w/v), 5 % fructose (w/v), varying concentrations for standard curves.
g) 20 µm sample injection volume.

Acids:

HPLC method (ion-exchange):

a) Mobile phase: 0.01 N H2SO4
b) Column: Phenomenex Rezex Organic Acid, 7.8x300 mm.
c) Temperature: 80 °C
d) Flow rate: 0.6 mL/min.
e) Detection: UV @ 214 nm.
f) 20 µm sample injection volume.

1.3.B.3 Moisture content

Moisture is determined by the following method:

Instrument: Sartorius MA 30 moisture analyzer.

Settings:

- 80 °C
- Automatic stop

Procedure:

- Tare an aluminum pan on the balance.
- Add about 1 g ShanStar to the pan. Disperse evenly.
- Analyze moisture for 5 minutes to "warm up" instrument.
- Tare a new aluminum pan on the balance.
- Add about 1 g ShanStar to the pan. Disperse evenly.
- Run automatic program until instrument stops determination.
- Repeat two more times.
- Average percent moisture results.

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1.3.C Sensitivity and Reproducibility of Methods

Sensitivity is given in terms of the maximum % deviation from the reported value. Reproducibility is given as the percentage of time that the measurement of the same sample has the same value.

Method	Sensitivity	Reproducibility
Crospovidone	+/- 10%	77%
Total Phenolics	+/- 17%	90%
Sugars and Acids	+/- 20%	90%
Water	+/-8%	95%

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Section 1.4 Summary of Manufacturing Process with Schematic

The manufacturing process produces a high quality stable juice and a powder that is saturated with bioflavonoids and polyphenolics from cranberry. The process schematic (Section 1.4.A) is found on page 18 at the end of this section. Resins of polyamide type, such as PVPP, are used in wine, beer and juice industries to remove precipitated and condensed polyphenols. Reasons for removing these condensed polyphenols are:

- Prevention of colloidal haze and development of sediments
- Color and clarity improvements due to the removal of off-colors produced in so-called browning reactions.
- Taste improvement resulting from the reduction of the excess of astringency caused by tannin compounds.

Most phenolic compounds retained in the spent resin are anthocyanins, catechins, and proanthocyanidins, which are highly beneficial to health. Some benefits include antibacterial, antiviral, antioxidant, and other phytochemical properties. Currently, these resins are discarded or recycled. This manufacturing process (patent pending) properly dries the saturated resin to a powder form so that the phenolic compounds remain bound to the resin. This powder can be used as an ingredient for dietary supplements, foods, drugs, and cosmetics.

The product is made by the following manufacturing steps:

- Juice is extracted from cranberries that are rich in natural phenolic compounds.
- After the juice extraction, the juice is de-fined to remove the undesired highly polymerized fractions of polyphenols.
- Then, properly filtered to ensure that all solids, bacteria and yeast are adequately removed.

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[redacted]

- Crospovidone (USP grade) is added and mixed thoroughly with the de-fined and filtered juice. The mixture is filtered to yield a clear, highly stable juice and a filtrate, a wet cake consisting mostly of the cranberry bioflavonoids and/or phenolic compounds bound to Crospovidone.
- The wet cake is then dried to contain approximately 10% water.

Referring to Section 1.4.A (p. 18 of this section), an apparatus 10 that manufactures a highly stable juice and a powder product saturated with phenolic compounds is shown. The apparatus includes a first tank (1), several connecting tubes (2, 4, 6, 8, 10, 12, 14, 16), a first pump (3), a first mixing tank (5), a second pump (7), a first plate & frame filter (9), a second mixing tank (11), a third pump (13), a second plate and frame filter (15), a juice receiving tank (17), a receiving pan (19), a drying apparatus (21), a spectrophotometer (22), and a comparator (23).

The tank (1) contains cranberry juice (A), extracted by conventional method. A portion of the juice (A) is transferred to the mixing tank (5) through the pump (3) and interconnecting tubes (2,4). A predetermined amount of gelatin (G) and bentonite (B) are added in the mixing tank (5). The amount and ratio of gelatin (G) and bentonite (B) depend on the type of juice and level of polymerized polyphenols. Filtering aids (FA) such as diatomaceous earth is then added in the mixing tank (5). The mixed solution (A1) is then filtered through a plate & frame filter (9) by the pump (7) and interconnecting tubes (6,8) to less than 3 NTU (Nephelos Turbidity Units). At this point, the de-fining and filtration operation is completed in the juice (A). Most of highly polymerized forms of polyphenols, and any bacteria and yeast cells naturally present in the juice (A) are removed at this stage.

The mixing tank (11) receives the de-fined and filtered juice (A) and is mixed with a predetermined ratio of Crospovidone (USP grade). The plate & frame filter (15) filters the mixed solution (A2). At the end of this filtration process, the remaining juice in the filtrate is pushed out with an inert gas such as nitrogen and stored in the tank (17). The

Crospovidone resin with the adsorbed bioflavonoids (A4) is collected in the collecting pan (19). The amount of bioflavonoids adsorbing to the Crospovidone can be controlled automatically by the use of preset limits for juice color, measured spectrophotometrically.

The wet cake adsorbed with phenolic compounds (A4) has a high moisture content ranging between 50% to 75%. The wet cake (A4) is dried to less than 10% moisture content. Preferably, the drier is a vacuum drier (21). At ~10% moisture, the product (A4) is very stable and has a shelf life of at least 1-year at room temperature.

Section 1.4.A Process Schematic.

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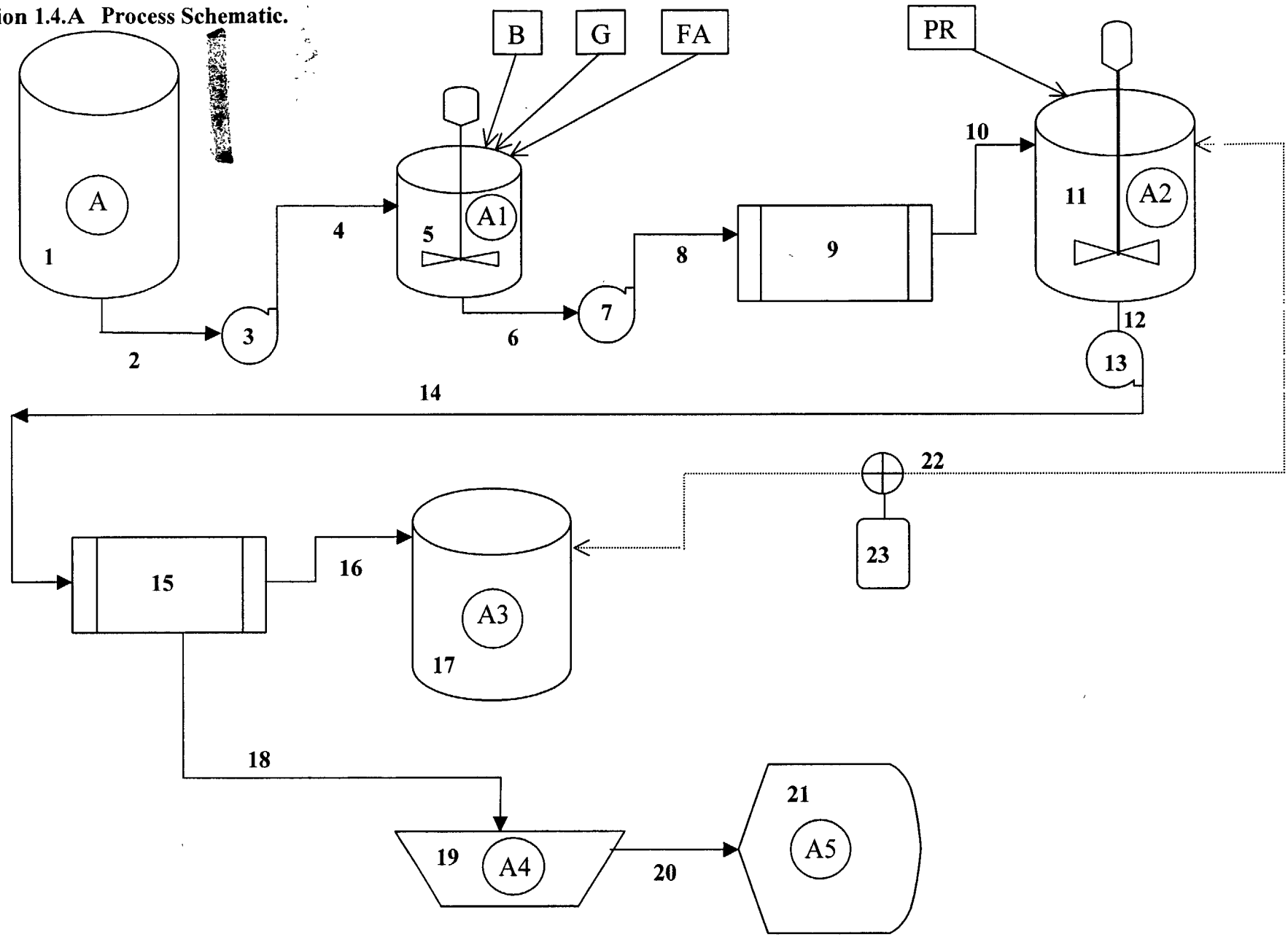

Section 2 Uses of ShanStar Cranberry

2.1 Proposed Uses

2.2 Past Use in Foods, Beverages and Dietary Supplements

2.3 First Date of Commercial Availability

2.4 Literature references for Cranberry, Crospovidone and Povidone

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Section 2.1 Proposed Uses

ShanStar Cranberry is being proposed for use as:

1) an additive to foods and beverages for special dietary use,
2) an additive to regular beverages.

In foods for special dietary use, ShanStar Cranberry will be used as a condiment or spice e.g. mixed together with cinnamon and powdered sugar it can be sprinkled on puddings, cereals and desserts. In beverages for special dietary use, ShanStar Cranberry will be mixed with thickened beverages for persons who have difficulty to drink normally e.g. stroke victims and other persons with partial paralysis of the facial muscles.

The specific benefits ShanStar Cranberry brings to these applications are:

1) Provision of natural bioflavonoids and plant polyphenolics which act to support a healthy urinary tract without the fiber, sugars and acids normally found in fruit and fruit juices.
2) Availability of plant polyphenolics in a format which is edible and digestible by aged or handicapped persons who are residents in longterm care facilities

The minimum and maximum proposed levels of use are listed below.

Proposed use	Minimum level	Maximum level	Estimated daily exposure
Beverage for special dietary use	None	0.5 mg per ml	240 mg in 480 ml (4 x 120 ml servings)
Food for special dietary use	None	0.5 mg per g	300 mg in 600 g of solid food (3 x 200 g servings)
Regular beverages	None	0.1 mg/ml	150 mg in 1500 ml (4 x 375 ml servings)

Section 2.2 Past use in Foods, Beverages and Dietary Supplements

Cranberry phenolics and/or Crospovidone and Povidone already exist in cranberries, various dietary supplements, OTC drugs and foods and beverages containing cranberries, cranberry juice or cranberry powder.

2.2.A In Beverages

Cranberry phenolics

Cranberry juice cocktail (27%) contains the same phenolics (See Section 4.2) as those found in ShanStar Cranberry extract. The recommended use of cranberry cocktail is at least 8 ounces per day, which, in the amount of total phenolics, is equivalent to approximately 540 mg of ShanStar Cranberry extract. People often drink 2 to 3 – 10 oz servings of cranberry juice per day. This is equivalent to the polyphenolics in 1080 to 1620 mg of ShanStar Cranberry.

Crospovidone-NF

In CFR 21, vol 3, part 175.105, "Additives used as adhesives for packaging in contact with food", **Povidone/PVP and its related homo- and co-polymers** (*one of which is PVPP or Crospovidone*) **are considered equivalent**. In this application, The ADI of Povidone will be cited for Crospovidone since they are considered equivalent in the Federal Code of Regulations.

Crospovidone is listed in the US Pharmacopeia- National Formulary and has a DMF, Type V-FDA Accepted Reference Information, #2297, June 14, 1974, on file with the FDA. Also its use in foods and beverages is limited only by Good Manufacturing Practice. Crospovidone-NF is approved for use in foods and beverages in Japan (Section 2.4.B, Number 7). Crospovidone is declared GRAS for use as a clarifying and stabilizing agent in beverage processing (21 CFR 173.50). The ADI of Povidone, the non-crosslinked version of Crospovidone, is 50 mg per kg of body weight (Section 2.4.B, Number 1). Since Povidone and Crospovidone are considered equivalents by CFR, [redacted]

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Crospovidone also has an ADI of 50 mg per kg of body weight. This translates to an ADI of ~3410 mg per day for a person weighing 68 kg.

2.2.B In Food

Cranberry Phenolics

Cranberries contain the same phenolics (See Section 4.2) as those found in ShanStar Cranberry extract. Numerous food products are on the market which contain cranberries, e.g. dried cranberries, granola, muffins, cereals, cookies, energy bars, etc. These foods are marketed as high in anti-oxidants, which act to slow the aging process and help maintain good general health.

Crospovidone-NF

In CFR 21, vol 3, part 175.105, "Additives used as adhesives for packaging in contact with food", **Povidone/PVP and its related homo- and co-polymers** (*one of which is PVPP or Crospovidone*) **are considered equivalent**. In this application, The ADI of Povidone will be cited for Crospovidone since they are considered equivalent in the Federal Code of Regulations. Crospovidone is listed in the US Pharmacopeia- National Formulary and has a DMF, Type V, FDA Accepted Reference Information, #2297, June 14, 1974, on file with the FDA. Also its use in foods and beverages is limited only by Good Manufacturing Practice. Crospovidone-NF is approved for use in foods and beverages in Japan (Section 2.4.B, Number 7). Crospovidone is declared GRAS for use as a clarifying and stabilizing agent in beverage processing (21 CFR 173.50). The ADI of Povidone, the non-crosslinked version of Crospovidone, is 50 mg per kg of body weight (Section 2.4.B, Number 1). Since Povidone and Crospovidone are considered equivalents by CFR, Crospovidone also has an ADI of 50 mg per kg of body weight. This translates to an ADI of ~3410 mg per day for a person weighing 68 kg.

2.2.C In Dietary Supplements

Cranberry Phenolics

Cranberry powder, which contains identical phenolics to those found in ShanStar Cranberry extract, is presently used in numerous dietary supplements as an antioxidant and in the maintenance of a healthy urinary tract function. Its current recommended level of use in powdered cranberry concentrate varies from 300 mg to 6.0 grams per day. In terms of total phenolics, 300 mg and 6 grams of cranberry powder are equivalent to approximately 32 mg and 640 mg of ShanStar Cranberry extract, respectively.

Crospovidone-NF

In CFR 21, vol 3, part 175.105, "Additives used as adhesives for packaging in contact with food", **Povidone/PVP and its related homo- and co-polymers** (*one of which is PVPP or Crospovidone*) **are considered equivalent**. In this application, The ADI of Povidone will be cited for Crospovidone since they are considered equivalent in the Federal Code of Regulations. Crospovidone is listed in the US Pharmacopeia- National Formulary and has a DMF, Type V-FDA Accepted Reference Information, #2297, June 14, 1974, on file with the FDA. Also its use in foods and beverages is limited only by Good Manufacturing Practice. Crospovidone-NF is approved for use in foods and beverages in Japan (Section 2.4.B, Number 7). Crospovidone is declared GRAS for use as a clarifying and stabilizing agent in beverage processing (21 CFR 173.50). The ADI of Povidone, the non-crosslinked version of Crospovidone, is 50 mg per kg of body weight (Section 2.4.B, Number 1). Since Povidone and Crospovidone are considered equivalents by CFR, Crospovidone also has an ADI of 50 mg per kg of body weight. This translates to an ADI of ~3410 mg per day for a person weighing 68 kg.

Crospovidone-NF or PVPP is currently used in numerous vitamin products and dietary supplements as a disintegrant. Its level of use varies from approximately 5 to 25 mg per tablet. Popular products in which it is listed as an ingredient include Centrum, Centrum Silver, Theragram.

Section 2.3 First Date of Commercial Availability

ShanStar Cranbery extract shipped to International Vitamin Company, Portland , Oregon. John McCarthy, IVC's marketing manager, confirmed that a dietary supplement containing ShanStar Cranberry extract was first sold by Costco during the week of June 6, 1999.

Currently being sold as a dietary supplement by:

- Costco
- RP Scherer
- Leiner
- Whole Foods
- Kirkman Laboratories
- International Vitamin Company

Section 2.4 Literature references for Cranberry, Crospovidone and Povidone

2.4.A Cranberry References

Most of the references in this section support cranberry phenolics as components of ShanStar Cranberry extract, the proposed additive to foods and beverages for special dietary use and as an additive to regular beverages.

Identification, quantification and functionality of the various phenolics is referred to in references 2-4, 16, 25, 37, 40-44.

Claimed benefits of this food additive are anti-microbial properties (references 7, 28, 31, 38, 39), anti-oxidant properties (references 10-12, 15), inhibition of coaggregation of bacteria and effect on dental plaque (references 9, 27), maintenance of a healthy urinary tract function (references 1, 5, 6, 8, 13, 14, 17-23, 26, 30-36), and possible beneficial effect on circulatory function (references 15, 24, 29).

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2. Petrus,-D.R., Visible and ultraviolet absorption and flourescence spectral characteristics of apple juice, cranberry juice cocktail, tomato and grape juices, grape jelly and strawberry preserves.
Adulteration of fruit juice beverages / edited by Steven Nagy, John A. Attaway, Martha E. Rhodes. New York : M. Dekker, c1988. p. 195-211.

3. Sapers,-G.M.; Hargrave,-D.L. Proportions of individual anthocyanins in fruits of cranberry cultivars. J-Am-Soc-Hortic-Sci. Alexandria, Va. The Society. Jan 1987. v. 112 (1) p. 100-104.

4. Madhavi, DL; Smith, MAL; Berger-Jimenez, D. Anthocyanins from cell cultures of cranberry, First International Symposium on Natural Colors for Foods, Nutraceuticals,

Beverages and Confectionery, Amherst, MA (USA),7-10 Nov 1993. (World Meeting Number 934 5030)

5. Avorn, J; Monane, M; Gurwitz, JH; Glynn, RJ; Choodnovskiy, I;
Lipsitz, LA, Journal of the American Medical Association [J. AM. MED. ASSOC.],vol. 271, no. 10, pp. 751-754, 1994.
Abstract
The objective of this study was to determine the effect of regular intake of cranberry juice beverage on bacteriuria and pyuria in elderly women. The subjects were a volunteer sample of 153 elderly women (mean age, 78.5 years). Subjects were randomly assigned to consume 300 mL per day (10 ounces) of a commercially available standard cranberry beverage or a specially prepared synthetic placebo drink that was indistinguishable in taste, appearance, and vitamin C content but lacked cranberry content. A baseline urine sample and six clean-voided study urine samples were collected at approximately 1-month intervals and tested quantitatively for bacteriuria and the presence of white blood cells. The findings suggest that use of a cranberry beverage reduces the frequency of bacteriuria with pyuria in older women. Prevalent beliefs about the effects of cranberry juice on the urinary tract may have microbiologic justification.

6. Sobota, AE. Inhibition of bacterial adherence by cranberry juice: Potential use for the treatment of urinary tract infections.
Journal of Urology [J. UROL.], vol. 131, no. 5, pp. 1013-1017,1984.
Abstract
Cranberry juice has been widely used for the treatment and prevention of urinary tract infections and is reputed to give symptomatic relief from these infections. Attempts to account for the potential benefit derived from the juice have focused on urine acidification and bacteriostasis. In this investigation it is demonstrated that cranberry juice is a potent inhibitor of bacterial adherence. A total of 77 clinical isolates of Escherichia coli were tested. Cranberry juice inhibited adherence by 75 per cent or more in over 60 per cent of the clinical isolates. Cranberry cocktail was also given to mice in the place of their normal water supply for a period of 14 days. Urine collected from these mice inhibited adherence of E. coli to uroepithelial cells by approximately 80 per cent.

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Abstract
An ethanolic extract of Cranberry exerted a significant antimicrobial effect on Saccharomyces bayanus and Pseudomonas fluorescens . The antimicrobial properties of cranberries were due to a number of factors. The results showed that proanthocyanidins provided 21.3% of the inhibition, the flavonols 18.5% and benzoic acid 15.6%.

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17. WINSLOW EH. SUPPORT FOR FOLK WISDOM ON CRANBERRY JUICE. AMERICAN JOURNAL OF NURSING, MAY, 1995, V95(N5):69-69.

18. HOPKINS WJ, HEISEY DM, JONLER M, UEHLING DT. REDUCTION OF BACTERIURIA AND PYURIA USING CRANBERRY JUICE. JAMA-JOURNAL OF THE AMERICAN MEDICAL ASSOCIATION, AUG 24,1994, V272(N8):588-589.

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Abstract

Dental plaque stability depends on bacterial adhesion to acquired pellicle, and on interspecies adhesion (or coaggregation). A high-molecular-weight cranberry constituent at 0.6 to 2.5 milligrams per milliliter reversed the coaggregation of 49 (58 percent) of 84 coaggregating bacterial pairs tested. It acted preferentially on pairs in which one or both members are gram-negative anaerobes frequently involved in periodontal diseases. Thus, the anticoaggregating cranberry constituent has the potential for altering the subgingival microbiota, resulting in conservative control of gingival and periodontal diseases.

However, the high dextrose and fructose content of the commercially available cranberry juice makes it unsuitable for oral hygiene use, and the beneficial effect of the high-molecular-weight constituent requires animal and clinical studies.

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Abstract

Vaccinium macrocarpon--the American cranberry--irreversibly inhibits the expression of P-fimbriae of E. coli. Further effects on the function and expression of P-fimbriae were studied by growing P-fimbriated E. coli in solid media laced with cranberry juice.

29. Wilson, T, Porcari, JP, Harbin, D. Cranberry extract inhibits low density lipoprotein oxidation. Life Sciences, 1998, 62(24):PL381-6.

Abstract

Cranberry juice consumption is often used for the treatment of urinary tract infections, but the effect of cranberry juice on heart disease has not been investigated. We evaluated how a cranberry extract containing 1,548 mg gallic acid equivalents/liter (initial pH=2.50) affected low density lipoprotein (LDL) oxidation induced by 10 micromolar cupric sulfate. When LDL oxidation took place in the presence of diluted cranberry extracts, the formation of thiobarbituric acid reactive substances (TBARS) and LDL electrophoretic mobility were reduced. LDL electrophoretic migration was also reduced when the cranberry extract had a pH of 7.00 prior to dilution. This study suggests that cranberry extracts have the ability to inhibit the oxidative modification of LDL particles.

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Cranberry concentrate: UTI prophylaxis [letter]. Journal of Family Practice, 1997 Aug, 45(2):167-8.

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32. Avorn, J. The effect of cranberry juice on the presence of bacteria
and white blood cells in the urine of elderly women. What is the role of bacterial adhesion?.Advances in Experimental Medicine and Biology, 1996, 408:185-6.

33. Nazarko, L. Infection control. The therapeutic uses of cranberry juice. Nursing Standard, 1995 May 17-23, 9(34):33-5.

Abstract

This article considers the treatment of recurrent cystitis. It examines the evidence for drinking cranberry juice to prevent recurrent urinary tract infections and discusses the nursing issues raised, drawing conclusions from the evidence presented.

34. Foxman, B, Geiger, AM, Palin, K, Gillespie, B, Koopman, JS, First-time urinary tract infection and sexual behavior. Epidemiology, 1995 Mar, 6(2):162-8.

Abstract

We studied the relation between sexual and health behaviors of women and first-time urinary tract infection (UTI). The study population was women using a university health service who were unmarried, had no UTI history, and who had engaged in sexual activity at least once. We found 86 cases of UTI, defined as one or more urinary symptoms and > or = 1,000 colony-forming units per ml urine of a known pathogen. We randomly sampled 288 controls from the student body. Vaginal intercourse increased the risk of UTI; this risk was further increased with condom use. After adjusting for vaginal intercourse with other birth control methods and recentness of current sexual partnership, a single sex act with a condom in the past 2 weeks increased UTI risk by 43%. Having a sex partner for less than 1 year vs 1 year or more, after adjustment for frequency of vaginal intercourse and birth control method, was associated with about twice the risk of UTI [odds ratio (OR) = 1.97; 95% confidence interval (CI) = 1.04-3.74]. After adjusting for frequency of vaginal intercourse, regular drinking of cranberry juice was protective against UTI (OR = 0.48; 95% CI = 0.19-1.02), whereas drinking carbonated soft drinks appeared to be associated with increased risk (OR = 2.37; 95% CI = 0.75-7.81). Using deodorant sanitary napkins or tampons was associated with a slight increase in risk of UTI (OR = 1.51; 95% CI = 0.74-3.06). Blacks had five times greater risk of UTI than whites after adjusting for frequency of vaginal intercourse (OR = 5.2; 95% CI = 1.89-24.63). We observed only modest differences in health behavior between racial groups.

35. Tsukada, K, Tokunaga, K, Iwama, T, Mishima, Y, Tazawa, K, Fujimaki, M. Cranberry juice and its impact on peri-stomal skin conditions for urostomy patients. Ostomy/Wound Management, 1994 Nov-Dec, 40(9):60-2, 64, 66-8.

Abstract

In urostomy patients, peristomal skin problems are common and may stem from alkaline urine. Cranberry juice appears to acidify urine and has bacteriostatic properties, and is widely recommended for the reduction of urinary tract infections. Therefore, it is hypothesized that drinking cranberry juice might also prevent and/or improve skin complications for urostomy patients. To test this hypothesis, pH measurements of the skin around the stoma and of the urine of 13 urostomy patients were taken before and after instituting a regimen of drinking 160 to 320 g of cranberry juice each day for an average

period of six months. Results showed an improvement in skin condition from 6 patients with erythema, maceration or pseudoepithelial hyperplasia at the beginning of the study to 2 patients with maceration or PEH. The average pH of the urine taken from the patients' pouches decreased a statistically significant amount from 8.0 to 7.3 ($p = 0.0277$), yet unexpectantly, the average pH of the fresh urine increased a statistically significant amount from 5.8 to 6.2 ($p = 0.0178$). Other results were not statistically significant. The authors conclude that while drinking cranberry juice did not appear to acidify the urine as expected, improvements were still seen in the skin conditions of the study participants, suggesting that drinking cranberry juice does positively impact the incidence of skin complications for these patients.

36. Fleet, JC. New support for a folk remedy: cranberry juice reduces bacteriuria and pyuria in elderly women. Nutrition Reviews, 1994 May, 52(5):168-70.

Abstract

Cranberry juice has developed a following as a simple, nonpharmacologic means to reduce or treat urinary tract infections, yet the scientific basis for such a claim has been lacking. A new study suggests that bacterial infections (bacteriuria) and associated influx of white blood cells into the urine (pyuria) can be reduced by nearly 50% in elderly women who drink 300 mL of cranberry juice cocktail each day over the course of a 6-month study. The results of this study suggest that consumption of cranberry juice is more effective in treating than preventing bacteriuria and pyuria. Along with earlier reports on the ability of cranberry juice to inhibit bacterial adherence to urinary epithelial cells in cell culture, this new work suggests that drinking cranberry juice each day may be clinically useful. Additional work must be conducted, however, to more completely define the efficacy of cranberry juice.

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38. Zafriri, D, Ofek, I, Adar, R, Pocino, M, Sharon, N. Inhibitory activity of cranberry juice on adherence of type 1 and type P fimbriated Escherichia coli to eucaryotic cells. Antimicrobial Agents and Chemotherapy, 1989 Jan, 33(1):92-98.

Abstract

Inhibition of bacterial adherence to bladder cells has been assumed to account for the beneficial action ascribed to cranberry juice and cranberry juice cocktail in the prevention of urinary tract infections (A. E. Sobota, J. Urol. 131:1013-1016, 1984). We have examined the effect of the cocktail and juice on the adherence of Escherichia coli expressing surface lectins of defined sugar specificity to yeasts, tissue culture cells, erythrocytes, and mouse peritoneal macrophages. Cranberry juice cocktail inhibited the adherence of urinary isolates expressing type 1 fimbriae (mannose specific) and P fimbriae [specific for alpha-D-Gal(1----4)-beta-D-Gal] but had no effect on a diarrheal isolate expressing a CFA/I adhesin. The cocktail also inhibited yeast agglutination by purified type 1 fimbriae. The inhibitory activity for type 1 fimbriated E. coli was

dialyzable and could be ascribed to the fructose present in the cocktail; this sugar was about 1/10 as active as methyl alpha-D-mannoside in inhibiting the adherence of type 1 fimbriated bacteria. The inhibitory activity for the P fimbriated bacteria was nondialyzable and was detected only after preincubation of the bacteria with the cocktail. Cranberry juice, orange juice, and pineapple juice also inhibited adherence of type 1 fimbriated E. coli, most likely because of their fructose content. However, the two latter juices did not inhibit the P fimbriated bacteria. We conclude that cranberry juice contains at least two inhibitors of lectin-mediated adherence of uropathogens to eucaryotic cells. Further studies are required to establish whether these inhibitors play a role in vivo.

39. Schmidt, DR, Sobota, AE. An examination of the anti-adherence activity of cranberry juice on urinary and nonurinary bacterial isolates. Microbios, 1988, 55(224-225):173-81.

Abstract

In a previous investigation it was demonstrated that cranberry juice cocktail was able to inhibit adherence in 77 clinical isolates of Escherichia coli obtained from patients with diagnosed urinary tract infections. This work has been extended to include clinical isolates of E. coli, Proteus, Klebsiella, Enterobacter and Pseudomonas isolated from urine, sputum, wound and stool. Bacterial strains isolated from urine adhere in greater numbers to urinary tract epithelial cells than organisms isolated from sputum, stool and wound sources. E. coli, isolated from urine, adheres to urinary epithelial cells, in numbers three times greater than E. coli isolated from other clinical sources, and thus appears to represent a unique population of cells in terms of adherence. Cranberry juice cocktail and urine and urinary epithelial cells obtained after drinking the cocktail all demonstrate antiadherence activity against Gram-negative rods isolated from urine and other clinical sources. Drinking the cocktail may be useful in managing urinary tract infections in certain patients.

40. Herrmann-K , [Constituents of blueberries and cranberries.] ORIGINAL (NON-ENGLISH) TITLE: Ueber die Inhaltsstoffe von Heidelbeeren und Preiselbeeren. Industrielle-Obst--und-Gemueseverwertung, 81 (7) 218-223, 1996.

Abstract

This review of the composition of different spp. of blueberry and cranberry (e.g. Vaccinium myrtillus L., V. vitis idaea L., V. oxycoccus L., V. angustifolium Ait. and V. macrocarpon Ait.) covers general chemical constituents, with particular reference to minerals, trace elements, vitamins, carbohydrates, acids, anthocyanins, other phenols and flavour compounds.

41. Siciliano-AA, Cranberry, HerbalGram, 1996, No. 38, 51-54; 18 ref., 2 pl.

Abstract
The chemistry and nutrition, history and medicinal uses of cranberries (Vaccinium oxycoccus and V. macrocarpon) are briefly discussed, and a summary of clinical studies assessing the use of cranberry products in the treatment of urinary tract infections is presented in tabular form.

42. Duke, JA, Beckstrom-Sternberg, Dr. Duke's Phytochemical and Ethnobotanical Databases: Chemicals and their Biological activities in : Vaccinium macrocarpon AITON (Ericaceae) -- American cranberry, Cranberry, Large Cranberry, Phytochemical Database, USDA-ARS-NGRL, Beltsville, Maryland, March 1998.

43. JLB, INC. Partially purified cranberry anti-adhesion activity, US Patent 5525341, June 11, 1996.

Abstract
An extract prepared from plants of the genus Vaccinium, especially cranberries, which is enriched for an activity which inhibits bacterial adhesion to surfaces. The extract is also enriched for polyphenol and flavonoid compounds, lacks detectable amounts of simple sugars, has a very low content of benzoic acid relative to raw cranberries, and lacks significant amounts of anthocyanins. Methods for preparing and for using the extract are also disclosed.

44. JLB, INC. Cranberry extract and biologically active compounds derived therefrom, US Patent 5646178, July 8, 1997.

Abstract
Compounds isolated from plant materials of the genus Vaccinium, which have biological activity measurable as inhibition with adhesion of bacterial cells to surfaces, are described. The specific compounds include procyanidins, leucocyanin, and leucodelphinin, and flavonol glucosides including myrecitin-3-pyranoside. An exemplary procyanidin compound is a substituted epicatechin-catechin dimer or other polymer. Also described is an extract prepared from plants of the genus Vaccinium, especially cranberries, which is enriched for anti-adhesion activity. The extract is enriched for polyphenol and flavonoid compounds, lacks detectable amounts of simple sugars, has a very low content of benzoic acid relative to raw cranberries, and lacks significant amounts of anthocyanins. Methods for preparing and for using the extract are disclosed.

45. Eur J Clin Nutr 2000 May;54(5):405-8

Effects of blueberry and cranberry juice consumption on the plasma antioxidant capacity of healthy female volunteers.

Pedersen CB, Kyle J, Jenkinson AM, Gardner PT, McPhail DB, Duthie GG [redacted]

[redacted]

Technical University of Denmark, 2800-Lyngby, Copenhagen, Denmark.

Objective: To assess whether consumption of 500 ml of blueberry juice or cranberry juice by healthy female subjects increased plasma phenolic content and antioxidant capacity. Design: Latin square arrangement to eliminate ordering effects. After an overnight fast, nine volunteers consumed 500 ml of blueberry juice, cranberry juice or a sucrose solution (control); each volunteer participated on three occasions one week apart, consuming one of the beverages each time. Blood samples were obtained by venipuncture at intervals up to four hours after consumption of the juices. Urine samples were also obtained four hours after consuming the juice. Results: Consumption of cranberry juice resulted in a significant increase in the ability of plasma to reduce potassium nitrosodisulphonate and Fe(III)-2,4, 6-Tri(2-pyridyl)-s-triazine, these measures of antioxidant capacity attaining a maximum after 60-120 min. This corresponded to a 30% increase in vitamin C and a small but significant increase in total phenols in plasma. Consumption of blueberry juice had no such effects. Conclusion: The increase in plasma antioxidant capacity following consumption of cranberry juice could mainly be accounted for by an increase in vitamin C rather than phenolics. This also accounted for the lack of an effect of the phenolic-rich but vitamin C-low blueberry juice. Sponsorship: Funded by the Scottish Executive Rural Affairs Department and the Danish Government.

46. Cochrane Database Syst Rev 2000;2:CD001322

Cranberries for treating urinary tract infections.
Jepson RG, Mihaljevic L, Craig J
15 Blackwood Crescent, Edinburgh, UK, EH9 1QZ. ruthj@ibm.net

BACKGROUND: Cranberries (particularly in the form of cranberry juice) have been used widely for several decades for the prevention and treatment of urinary tract infections. The aim of this review is to assess the effectiveness of cranberries in treating such infections. OBJECTIVES: To assess the effectiveness of cranberries for the treatment of urinary tract infections. SEARCH STRATEGY: The search strategy developed by the Cochrane Renal Group was used. Also, companies involved with the promotion and distribution of cranberry preparations were contacted; electronic databases and the Internet were searched using English and non English language terms; reference lists of review articles and relevant trials were also searched. SELECTION CRITERIA: All randomised or quasi randomised controlled trials of cranberry juice or cranberry products for the treatment of urinary tract infections. Trials of men, women or children were included. DATA COLLECTION AND ANALYSIS: Titles and abstracts of studies that were potentially relevant to the review were screened by one reviewer, RJ, who discarded studies that were clearly ineligible but aimed to be overly inclusive rather than
risk losing relevant studies. Reviewers RJ and LM independently assessed whether the studies met the inclusion criteria. Further information was sought from the authors where

papers contained insufficient information to make a decision about eligibility. MAIN RESULTS: No trials were found which fulfilled all of the inclusion criteria. Two trials were excluded because they did not have any relevant outcomes. REVIEWER'S CONCLUSIONS: After a thorough search, no randomised trials which assessed the effectiveness of cranberry juice for the treatment of urinary tract infections were found. Therefore, at the present time, there is no good quality evidence to suggest that it is effective for the treatment of urinary tract infections. Well-designed parallel group, double blind trials comparing cranberry juice and other cranberry products versus placebo to assess the effectiveness of cranberry juice in treating urinary tract infections are needed. Outcomes should include reduction in symptoms, sterilisation of the urine, side effects and adherence to therapy. Dosage (amount and concentration) and duration of therapy should also be assessed. Consumers and clinicians will welcome the evidence from these trials.

47. JAMA 2000 Apr 5;283(13):1691

Does cranberry juice have antibacterial activity?
Lee YL, Owens J, Thrupp L, Cesario TC

48. Age Ageing 2000 Jan;29(1):9-12

What's the use of cranberry juice?
Harkins KJ , St James's University Hospital, Leeds, UK.

49. World J Urol 1999 Dec;17(6):359-63

Potential preventive strategies and therapies in urinary tract infection.
Reid G

Lawson Research Institute, London, Ontario, Canada. gregor@julian.uwo.ca

There are perhaps five strategies either presently advocated or under investigation for prevention of recurrent urinary tract infection (UTI): antibiotics, including natural peptides; functional foods; vaccines; probiotics; and miscellaneous, including avoidance of spermicides and maintenance of good hygiene. It is not possible to state the proportion of patients using antibiotics versus foods such as cranberry or using alternative approaches such as avoidance of spermicides. The majority of women who are referred to specialists will be prescribed long-term, low-dose antibiotics. However, given the magnitude of the problem, it is safe to state that large numbers of women are at least experimenting with alternative remedies such as drinking of cranberry juice or ingestion of herbal remedies with a view to enhancing their immune response. Vaccine development remains a long way from human use and has yet to be developed for organisms other than Escherichia coli. The use of probiotics of restore the normal vaginal

flora and provide a competitive bacterial barrier to pathogens is close to becoming available as an alternative preventive approach. The next decade should see the introduction of new methods for reduction of the high incidence of UTI and better management of recurring urogenital infections.

50. Lancet 1999 Feb 20;353(9153):673

Cranberry juice and prevention of recurrent urinary tract infection.

Kerr KG

2.4.B Crospovidone (Polyvinylpolypyrrolidone) and Povidone (Polyvinylpyrrolidone)

This section provides a list of references which establish the safety of Crospovidone as a food additive (reference 8), and an ADI for Povidone (reference 9), and its safety and effect as a food additive (references 12-17) and provides information which supports the safety of Crospovidone as a component of ShanStar Cranberry extract (reference 6,7, 9-11). Various studies which illustrate the mechanism of beverage stabilization and adsorption of phenolics and bioflavonoids by Crospovidone (PVPP) are also given (references 1-5).

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Abstract

Polyvinylpolypyrroridone (PVPP) has been used in many countries for removing polyphenols in beer and wine, and was permitted as a food additive in Japan in April

1995. Prior to this approval of the compound, we studied procedures for identifying PVPP and its water soluble substances by colorimetry and infrared (IR) spectrometry for the establishment of the specification.

8. WHO, Evaluation of certain food additives and contaminants, 27th Report of the Joint FAO/WHO Expert Committee on Food Additives, WHO Technical Report Series 696, 1983, WHO, Geneva Switzerland.

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stability of new vehicle. Chem Pharm Bull (Tokyo). 1999 Apr;47(4):492-7.

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Section 3. Method for detecting ShanStar Cranberry

3.1 Qualitative and quantitative method for determining ShanStar Cranberry in foods and beverages

3.2 Sensitivity and reproducibility of method

3.1 Qualitative and quantitative method for determining ShanStar Cranberry in foods and beverages

ShanStar Cranberry extract has a high content of polyphenolic compounds selectively extracted from cranberry juice. The manufacturing relies on the tendency of phenolic compounds from the juice to form complexes with Crospovidone. Complex formation is easily reversed with a change in pH, which naturally occurs during passage through the digestive system.

The present procedure was designed to qualitatively verify the presence of ShanStar in food products to which it may have been added. It consists in measuring the *phenolic complexing activity* (**PHCA**) remaining in the product after a proper treatment.
The sample to analyze is dried and then milled to a particle size of less than 0.5-mm diameter. It is then mixed with acid washed diatomaceous earth and submitted subsequently to a treatment with a series of alternating acidic and basic solvents. The solvents used were chosen for their ability to dissolve and oxidize compounds commonly found in food without significantly affecting the **PHCA** value of the Crospovidone fraction present in the product. By eliminating any other sources of **PHCA** except the Crospovidone in ShanStar Cranberry extract, it becomes possible to quantitate the amount present.

The results are expressed in terms of polyvinylpolypyrrolidone (PVPP, Crospovidone), an insoluble form of polyvinylpyrrolidone. A convenient standard curve is prepared using the proper amounts of this polymer.

This method gives acceptable results for determining ShanStar in other foods provided the sample preparation is careful and complete.

Equipment necessary:
Buchner funnel
Spectrophotometer
Centrifuge
100 ml volumetric flasks, etc.

Reagents:

1. Diatomatcous earth filter aid	(i.e. Celite 545)
2. Deionized water	
3. Hydrochloric acid	(1 normal)
4. Hydrochloric acid	(4 normal)
5. Hydrogen peroxide	(3% w/w)
6. Methanol	(HPLC grade)
7. Folin-Ciocalteu's phenol reagent	(2.0 normal, Sigma Chemical Co.)
8. Sodium Carbonate solution	(20% w/v)
9. Sodium hydroxide	(1 normal @ 180 °F)
10. Tannic acid solution	(1 mg/mL tannin in 1% citric acid solution)

Filter Precoat:

1. Wash 1 g of filter aid with 10 mL of 1 N HCl.
2. Add the filter aid to a 5 cm diameter Buchner filter funnel with a non gauze milk filter.
3. Add 25 mL water to the precoat and collect.
4. Using the eluent, again wash the precoat and collect. Only a minimal amount of filter aid will not be caught in the precoat. This water can be discarded.

Sample Preparation:

1. Wash 2 g of filter aid with 20 mL 1 N HCl.
2. Weigh out 500 mg finely ground sample and add to filter aid. Grind together to develop homogenous mixture.
3. Transfer to a 250 mL beaker, add 50 mL water and stir for 10 minutes. Use necessary volume of water for transfer of solids.
4. Transfer to centrifuge tubes and centrifuge at approximately 1200 rpm for 10 min.
5. Discard supernatant. ShanStar, when centrifuged properly, will collect at the bottom of the tube with the filter aid.
6. Transfer solids to precoated filter. Wash centrifuge tubes with water as necessary. Transfer with care to not unduly disrupt filter precoat. After addition of product, place another milk gauze over the filtrate to help prevent disturbances when adding solvents.

7. After filtration of water, add in succesion, as indicated by Table 1.

Table 1

water	100 mL
methanol	100 mL
hydrochloric acid (4 N)	25 mL
water	100 mL
methanol	100 mL
sodium hydroxide (180 °F)	100 mL
water	50 mL
sodium hydroxide (180 °F)	100 mL
hydrogen peroxide (3%)	25 mL
water	100 mL

8. When filtration complete, transfer entire filter cake to a 100 mL volumetric falsk. To the filtrate, add 50 mL tannic acid solution (1 mg/mL tannin in 1% citric acid) and bring to volume with water. Mix by inverting flask by hand for 15 minutes.
9. Centrifuge at 1200 rpm for 10 minutes.
10. Add 1 mL of supernatant to a 100 volumetric flask with 50 mL water. To the solution, add 5 mL Folin-Ciocalteu's reagent, 15 mL 20% sodium carbonate solution and bring to volume with water. Allow to develop color for 2 hours. Read absorbance at 760 nm versus a blank of Folin-Ciocalteu's and sodium carbonate prepared as above.

Control:

1. Wash 3 g filter aid with 30 mL 1 N HCl followed by 100 mL water.
2. When filtration complete, transfer entire filter cake to a 100 mL volumetric flask. To the filtrate, add 50 mL tannic acid solution (1 mg/mL tannin in 1% citric acid) and bring to volume with water. Mix by inverting flask by hand for 15 minutes.
3. Centrifuge at 1200 rpm for 10 minutes.
4. Add 1 mL of supernatant to a 100 volumetric flask with 50 mL water. To the solution, add 5 mL Folin-Ciocalteu's reagent, 15 mL 20% sodium carbonate solution and bring to volume with water. Allow to develop color for 2 hours. Read absorbance at 760 nm versus a blank of Folin-Ciocalteu's and sodium carbonate prepared as above.

Results:

The amounts of tannin remaining in solution for the sample and blank are determined. The difference corresponds to the tannin retained in the active sample solids. It provides a measurement of the phenolic complexing activity (**PHCA**) of the sample.

The amount of active polymer in the sample can be expressed in terms of PVPP by preparing an appropriate calibration curve.

Calculations:

Example:

Absorbance at 760 nm for

1-	control:	0.591
2-	sample:	0.462

A (760) = 0.591 correlates to 50 mg tannin and A (760) = 0.462 correlates to x mg tannin

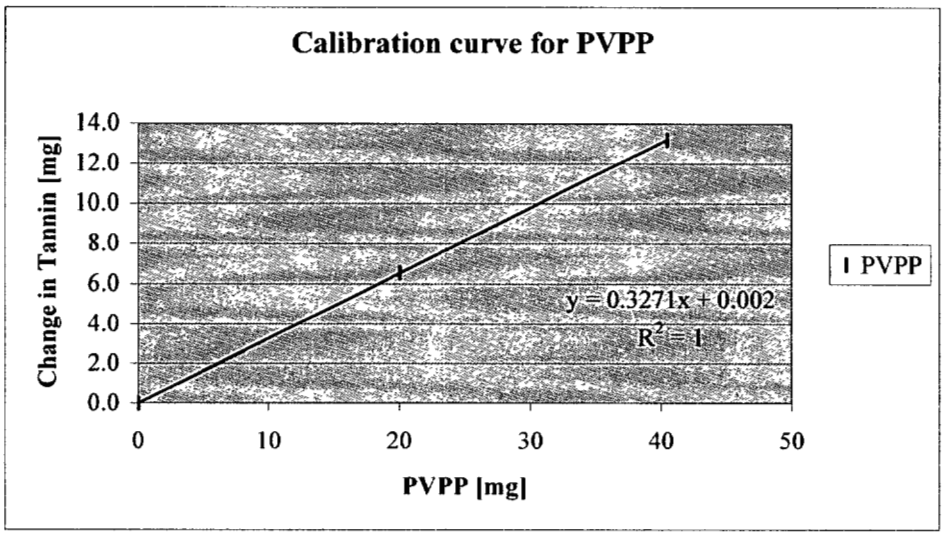
$$\frac{0.591}{0.462} = \frac{50}{x}$$

So, x = 39.1 mg tannin present in the tannin solution exposed to the sample and represents a change in tannin of 10.9 mg tannin. Therefore, treated sample solids retained 10.9-mg tannin. This number can then be plotted against the calibration curve to express in equivalent amounts of PVPP.

Calibration Curve:

1. A calibration curve must be prepared using adequate amounts of PVPP.
2. Create a calibration curve with PVPP samples as indicated above.

Figure 1



NOTE: **Results are for example purposes only**

Figure 1 represents a calibration curve of PVPP. Table 2 shows sample results for a calibration curve. Also shown in Table 2 are the results of a ShanStar Cranberry sample mixed with a Famous Amos™ Brand chocolate chip cookie.

[redacted] 000427

Table 2

PVPP [mg]	A (760 nm)	Tannin present after treatment [mg]	Change in Tannin [mg]
0.00	0.420	50.0	0.0
20.0	0.365	43.5	6.5
40.4	0.309	36.8	13.2
ShanStar Cranberry 39.6	0.340	40.5	9.5

The resulting number, based on the calibration curve, is 29 mg as PVPP.

Due to the nature of the procedure, the amount that can be adsorbed by the PVPP is limited. The change in tannin will level off as in Figure 3. It is therefore advised that the calibration curve should be appropriate for the amount of ShanStar in the product. For example, if only 25 mg of ShanStar per 500 mg of cookie is present, the use of 0, 10, 25, and 50 mg PVPP would be appropriate. If the product contains larger amounts of ShanStar then the calibration curve should be altered so that it reflects the higher level. This can be accomplished by doubling the amount of tannin solution and using a 200-ml volumetric flask. Appropriate alterations in the volumes of the reagents used for color development is acceptable.

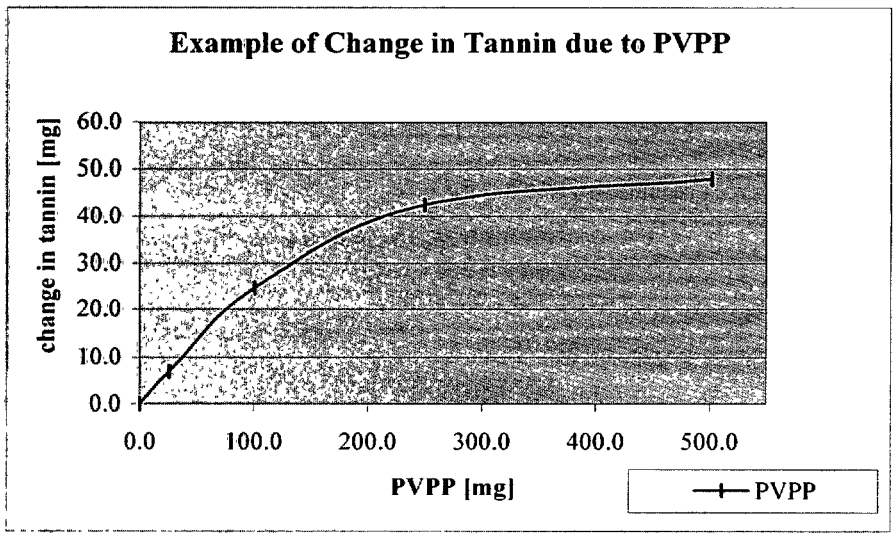


Figure 3

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Notes:

1. The solvents should elute through the samples easily. However, when the sodium hydroxide is added, degradation products form that tend to slow the elution rate. A small vacuum can be applied to help increase the elution rate.
2. The elution rate should be kept relatively constant for all the samples.
3. For accuracy the tannin in the solution brought in contact with the sample solids should not drop to less than half of the original.

3.2 Sensitivity and reproducibility of method

Sensitivity is given in terms of the maximum % deviation from the reported value. Reproducibility is given as the percentage of time that the measurement of the same sample has the same value.

The sensitivity and reproducibility of the method in Section 3.1 is:

1) Sensitivity	+/- 5%
2) Reproducibility	80%

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Section 4. Safety and functionality in Foods and Beverages

4.1 References to clinical studies on cranberry juice and solids

4.2 Evidence that ShanStar Cranberry phenolics are identical to GRAS counterpart of natural origin

4.3 Miscellaneous safety data

4.3.A Summary of studies on Allowable Daily Intake (ADI) of PVPP cited by JECFA/FAO/WHO

4.3.B Specific phenolics – Quercetin, Myricetin and Kaempferol

4.3.C References on PVP and PVPP from Toxline Database

4.3.D Expert opinion on safety of Crospovidone NF as an additive to foods and beverages

4.4 Adverse effects and/or consumer complaints

4.5 Stability data and proposed expiration date

4.6 MSDS for ShanStar Cranberry extract

4.7 MSDS for Polyvinylpolypyrrolidone

4.8 Evaluation of dietary exposure

4.1 References to clinical studies of cranberry juice and cranberry fruit solids

ShanStar Cranberry extract has not been tested clinically. However the plethora of published works on the use of cranberry juice, cocktail (references 2-29) and dried, cranberry fruit solids (reference 1) supports the beliefs that the phenolics from cranberry are effective in maintaining a healthy urinary tract, decreasing low density lipoproteins and reducing the formation of dental plaque. Recent studies have also identified the active components of cranberry.

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Abstract

The objective of this study was to determine the effect of regular intake of cranberry juice beverage on bacteriuria and pyuria in elderly women. The subjects were a volunteer sample of 153 elderly women (mean age, 78.5 years). Subjects were randomly assigned to consume 300 mL per day of a commercially available standard cranberry beverage or a specially prepared synthetic placebo drink that was indistinguishable in taste, appearance, and vitamin C content but lacked cranberry content. A baseline urine sample and six clean-voided study urine samples were collected at approximately 1-month intervals and tested quantitatively for bacteriuria and the presence of white blood cells. The findings

suggest that use of a cranberry beverage reduces the frequency of bacteriuria with pyuria in older women. Prevalent beliefs about the effects of cranberry juice on the urinary tract may have microbiologic justification.

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Journal of Urology [J. UROL.], vol. 131, no. 5, pp. 1013-1017,1984.

Abstract

Cranberry juice has been widely used for the treatment and prevention of urinary tract infections and is reputed to give symptomatic relief from these infections. Attempts to account for the potential benefit derived from the juice have focused on urine acidification and bacteriostasis. In this investigation it is demonstrated that cranberry juice is a potent inhibitor of bacterial adherence. A total of 77 clinical isolates of Escherichia coli were tested. Cranberry juice inhibited adherence by 75 per cent or more in over 60 per cent of the clinical isolates. Cranberry cocktail was also given to mice in the place of their normal water supply for a period of 14 days. Urine collected from these mice inhibited adherence of E. coli to uroepithelial cells by approximately 80 per cent.

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Abstract

Dental plaque stability depends on bacterial adhesion to acquired pellicle, and on interspecies adhesion (or coaggregation). A high-molecular-weight cranberry constituent at 0.6 to 2.5 milligrams per milliliter reversed the coaggregation of 49 (58 percent) of 84 coaggregating bacterial pairs tested. It acted preferentially on pairs in which one or both members are gram-negative anaerobes frequently involved in periodontal diseases. Thus, the anticoaggregating cranberry constituent has the potential for altering the subgingival microbiota, resulting in conservative control of gingival and periodontal diseases. However, the high dextrose and fructose content of the commercially available cranberry juice makes it unsuitable for oral hygiene use, and the beneficial effect of the high-molecular-weight constituent requires animal and clinical studies.

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Abstract

Cranberry juice consumption is often used for the treatment of urinary tract infections, but the effect of cranberry juice on heart disease has not been investigated. We evaluated how a cranberry extract containing 1,548 mg gallic acid equivalents/liter (initial pH=2.50) affected low density lipoprotein (LDL) oxidation induced by 10 micromolar cupric sulfate. When LDL oxidation took place in the presence of diluted cranberry extracts, the formation of thiobarbituric acid reactive substances (TBARS) and LDL electrophoretic mobility were reduced. LDL electrophoretic migration was also reduced when the cranberry extract had a pH of 7.00 prior to dilution. This study suggests that cranberry extracts have the ability to inhibit the oxidative modification of LDL particles.

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Cranberry concentrate: UTI prophylaxis [letter]. Journal of Family Practice, 1997 Aug, 45(2):167-8.

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and white blood cells in the urine of elderly women. What is the role of bacterial adhesion?.Advances in Experimental Medicine and Biology, 1996, 408:185-6.

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Nursing Standard, 1995 May 17-23, 9(34):33-5.

Abstract

This article considers the treatment of recurrent cystitis. It examines the evidence for drinking cranberry juice to prevent recurrent urinary tract infections and discusses the nursing issues raised, drawing conclusions from the evidence presented.

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Abstract

We studied the relation between sexual and health behaviors of women and first-time urinary tract infection (UTI). The study population was women using a university health service who were unmarried, had no UTI history, and who had engaged in sexual activity at least once. We found 86 cases of UTI, defined as one or more urinary symptoms and > or = 1,000 colony-forming units per ml urine of a known pathogen. We randomly sampled 288 controls from the student body. Vaginal intercourse increased the risk of UTI; this risk was further increased with condom use. After adjusting for vaginal intercourse with other birth control methods and recentness of current sexual partnership, a single sex act with a condom in the past 2 weeks increased UTI risk by 43%. Having a sex partner for less than 1 year vs 1 year or more, after adjustment for frequency of vaginal intercourse and birth control method, was associated with about twice the risk of UTI [odds ratio (OR) = 1.97; 95% confidence interval (CI) = 1.04-3.74]. After adjusting for frequency of vaginal intercourse, regular drinking of cranberry juice was protective against UTI (OR = 0.48; 95% CI = 0.19-1.02), whereas drinking carbonated soft drinks appeared to be associated with increased risk (OR = 2.37; 95% CI = 0.75-7.81). Using deodorant sanitary napkins or tampons was associated with a slight increase in risk of UTI (OR = 1.51; 95% CI = 0.74-3.06). Blacks had five times greater risk of UTI than whites after adjusting for frequency of vaginal intercourse (OR = 5.2; 95% CI = 1.89-24.63). We observed only modest differences in health behavior between racial groups.

25. Tsukada, K, Tokunaga, K, Iwama, T, Mishima, Y, Tazawa, K, Fujimaki, M. Cranberry juice and its impact on peri-stomal skin conditions for urostomy patients. Ostomy/Wound Management, 1994 Nov-Dec, 40(9):60-2, 64, 66-8.

Abstract

In urostomy patients, peristomal skin problems are common and may stem from alkaline urine. Cranberry juice appears to acidify urine and has bacteriostatic properties, and is widely recommended for the reduction of urinary tract infections. Therefore, it is hypothesized that drinking cranberry juice might also prevent and/or improve skin complications for urostomy patients. To test this hypothesis, pH measurements of the skin around the stoma and of the urine of 13 urostomy patients were taken before and after instituting a regimen of drinking 160 to 320 g of cranberry juice each day for an average period of six months. Results showed an improvement in skin condition from 6 patients with erythema, maceration or pseudoepithelial hyperplasia at the beginning of the study to 2 patients with maceration or PEH. The average pH of the urine taken from the patients' pouches decreased a statistically significant amount from 8.0 to 7.3 ($p = 0.0277$), yet unexpectantly, the average pH of the fresh urine increased a statistically significant amount from 5.8 to 6.2 ($p = 0.0178$). Other results were not statistically significant. The authors conclude that while drinking cranberry juice did not appear to acidify the urine as expected, improvements were still seen in the skin conditions of the study participants, suggesting that drinking cranberry juice does positively impact the incidence of skin complications for these patients.

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Abstract

Cranberry juice has developed a following as a simple, nonpharmacologic means to reduce or treat urinary tract infections, yet the scientific basis for such a claim has been lacking. A new study suggests that bacterial infections (bacteriuria) and associated influx of white blood cells into the urine (pyuria) can be reduced by nearly 50% in elderly women who drink 300 mL of cranberry juice cocktail each day over the course of a 6-month study. The results of this study suggest that consumption of cranberry juice is more effective in treating than preventing bacteriuria and pyuria. Along with earlier reports on the ability of cranberry juice to inhibit bacterial adherence to urinary epithelial cells in

cell culture, this new work suggests that drinking cranberry juice each day may be clinically useful. Additional work must be conducted, however, to more completely define the efficacy of cranberry juice.

27. Zafriri, D, Ofek, I, Adar, R, Pocino, M, Sharon, N. Inhibitory activity of cranberry juice on adherence of type 1 and type P fimbriated Escherichia coli to eucaryotic cells. Antimicrobial Agents and Chemotherapy, 1989 Jan, 33(1):92-98.

Abstract

Inhibition of bacterial adherence to bladder cells has been assumed to account for the beneficial action ascribed to cranberry juice and cranberry juice cocktail in the prevention of urinary tract infections (A. E. Sobota, J. Urol. 131:1013-1016, 1984). We have examined the effect of the cocktail and juice on the adherence of Escherichia coli expressing surface lectins of defined sugar specificity to yeasts, tissue culture cells, erythrocytes, and mouse peritoneal macrophages. Cranberry juice cocktail inhibited the adherence of urinary isolates expressing type 1 fimbriae (mannose specific) and P fimbriae [specific for alpha-D-Gal(1----4)-beta-D-Gal] but had no effect on a diarrheal isolate expressing a CFA/I adhesin. The cocktail also inhibited yeast agglutination by purified type 1 fimbriae. The inhibitory activity for type 1 fimbriated E. coli was dialyzable and could be ascribed to the fructose present in the cocktail; this sugar was about 1/10 as active as methyl alpha-D-mannoside in inhibiting the adherence of type 1 fimbriated bacteria. The inhibitory activity for the P fimbriated bacteria was nondialyzable and was detected only after preincubation of the bacteria with the cocktail. Cranberry juice, orange juice, and pineapple juice also inhibited adherence of type 1 fimbriated E. coli, most likely because of their fructose content. However, the two latter juices did not inhibit the P fimbriated bacteria. We conclude that cranberry juice contains at least two inhibitors of lectin-mediated adherence of uropathogens to eucaryotic cells. Further studies are required to establish whether these inhibitors play a role in vivo.

28. Schmidt, DR, Sobota, AE. An examination of the anti-adherence activity of cranberry juice on urinary and nonurinary bacterial isolates. Microbios, 1988, 55(224-225):173-81.

Abstract

In a previous investigation it was demonstrated that cranberry juice cocktail was able to inhibit adherence in 77 clinical isolates of Escherichia coli obtained from patients with diagnosed urinary tract infections. This work has been extended to include clinical isolates of E. coli, Proteus, Klebsiella, Enterobacter and Pseudomonas isolated from urine, sputum, wound and stool. Bacterial strains isolated from urine adhere in greater numbers to urinary tract epithelial cells than organisms isolated from sputum, stool and wound sources. E. coli, isolated from urine, adheres to urinary epithelial cells, in numbers three times greater than E. coli isolated from other clinical sources, and thus appears to represent a unique population of cells in terms of adherence. Cranberry juice cocktail and urine and urinary epithelial cells obtained after drinking the cocktail all demonstrate antiadherence activity against Gram-negative rods isolated from urine and other clinical sources. Drinking the cocktail may be useful in managing urinary tract infections in certain patients.

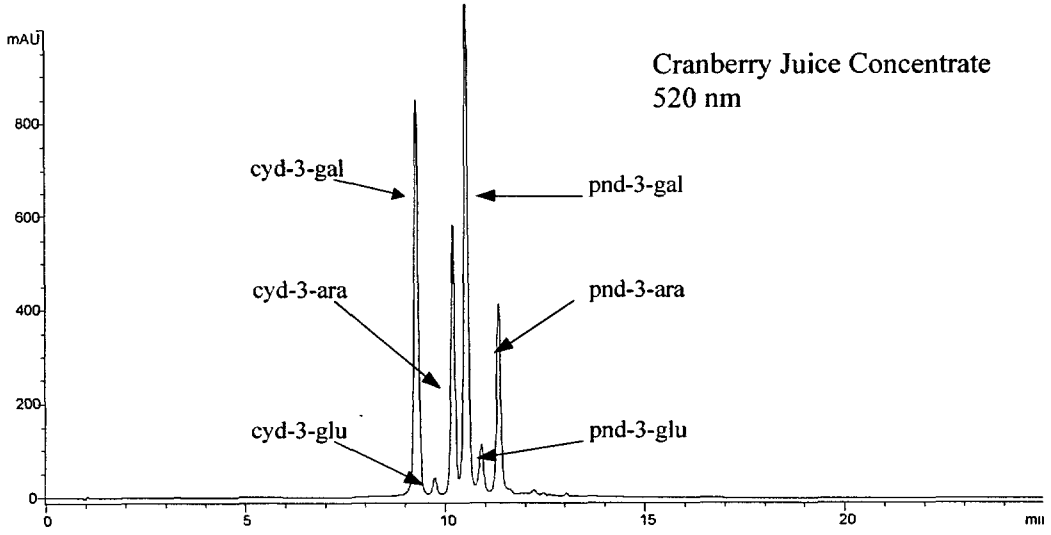
29. Siciliano-AA, Cranberry, HerbalGram, 1996, No. 38, 51-54; 18 ref., 2 pl.

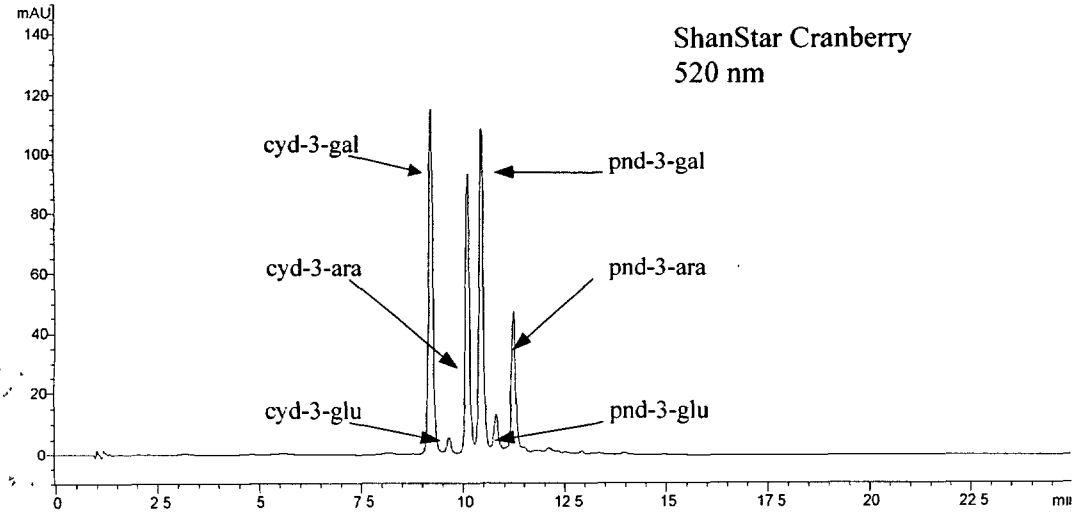
Abstract

The chemistry and nutrition, history and medicinal uses of cranberries (Vaccinium oxycoccus and V. macrocarpon) are briefly discussed, and a summary of clinical studies assessing the use of cranberry products in the treatment of urinary tract infections is presented in tabular form.

4.2 Evidence that ShanStar Cranberry phenolics are identical to GRAS counterpart of natural origin

The RP-HPLC Chromatographic profiles for pure cranberry juice and ShanStar Cranberry show identical peak profiles at 520 nm. This indicates that there is no change in the compounds due to processing. These peaks represent some of the anthocyanins present in cranberry. See Reference 46 in section 2.4.A to see list of cranberry components and function.





[redacted]

4.3 Miscellaneous safety data

4.3.A Summary of studies on Allowable Daily Intake (ADI) of PVPP cited by JECFA/FAO/WHO

"A Submission by International Specialty Products (Wayne, NJ, USA) to the Joint Expert Committee on Food Additives of the FAO/WHO on the safety of PVPP and to establish an Allowable Daily Intake"

Various studies conducted by ISP are summarized below. More complete information is available from International Specialty Products, Wayne, NJ, USA and from the **Drug Master File # 2297 on file with the FDA**.

ACUTE ORAL TOXICITY

Rats and mice were fed 5g/kg (bodyweight) POLYVINYLPOLYPYRROLIDONE. No abnormal symptoms or deaths were observed after 24, 48 or 72 hours.

28-DAY FEEDING STUDY IN RATS:

Five groups of 40 rats each (equally divided by sex) were dosed for 4 weeks at 0, 1, 2.5, 5 or 10% PVPP in their feed followed by 2 weeks of no PVPP in the diet. At the 10% level, a slight bodyweight decrease was observed. Dose-dependent urinary casts in the urine sediment of females were reversible when PVPP feeding stopped. Clinical chemistry and hematology, necropsy results and observations showed no differences from control animals. PVPP was not deposited in the small intestine mucosa or mesenteric lymph nodes.

90-DAY FEEDING STUDY IN RATS:

Three groups of 40 Wistar strain rats (equally divided by sex) were fed diets for 90 days at 0, 2, or 10% PVPP. No compound related effects were noted in behavior, appearance, food consumption, feed efficacy, bodyweight gain, clinical studies (hematology, clinical biochemistry and urinalysis), necropsy or histopathology results.

28-DAY FEEDING STUDY IN DOGS:

Three groups of 6 beagle dogs were dosed by stomach tube with an aqueous suspension of PVPP at 1000, 2000 or 5050 mg/kg bodyweight for 4 weeks.Control animals received water or a cellulose suspension (400 mg/kg bodyweight). No compound related effects were noted in food and water consumption, hematology, clinical chemistry, urinalysis and histopathology. No accumulation of PVPP was found in the liver, kidneys or mesenteric lymph nodes.

180-DAY FEEDING STUDY IN DOGS:

Three groups of 8 beagle dogs were dosed by stomach tube with an aqueous suspension of 300, 1200 or 4888 mg/kg bodyweight for 26 weeks. Control animals received water or a cellulose suspension (4800 mg/kg bodyweight). Because the high dose level dogs showed increasing emesis during weeks 7 - 12, divided doses were given. No compound related effects were noted in behavior, food and water consumption, growth, hematology, clinical chemistry or urinalysis results, electrocardiography, ophthalmology, auditory tests, gross or histopathology. No PVPP deposition or storage was found in the liver, kidneys or mesenteric lymph nodes.

TERATOGENICITY

Three groups of 26 pregnant SPF rats were dosed by gavage with suspensions of PVPP (0, 1000 or 3000 mg/kg bodyweight) in carboxymethyl cellulose from day 6 to 15 and sacrificed on day 20 of gestation. One group was left untreated.A slight reversible decrease in weight gain occurred in the high-dose group and the weights of the fetuses of all test animals were slightly lower than of controls.No changes in fetal length were observed.There were no significant clinical symptoms, maternal deaths and no compound-related necropsy findings for the dams.Conception rate and live and dead implantations were not affected.The fetuses displayed no compound-related skeletal or visceral abnormalities.

Two additional groups of 12 pregnant SPF rats received the same dose levels (1000 or 3000 mg/kg) from day 15 to day 21 post-parturition.Weight gain, mortality, delivery time and litter size of the test animals were comparable to the control.No macroscopic compound-related changes were observed in the mothers.Pups of treated animals showed similar mortality, bodyweight and behavior as the control.Autopsy revealed no compound-related skeletal or visceral abnormalities.Effects noted were lower heart weight of male and female pups and lower spleen weight of male pups in the 3000 ppm (0.3%) group, as well as some changes in the males' kidneys and females' livers.No other compound-related effects were noted in the dams or pups.

PHARMACOKINETICS

Studies on ^{14}C-labeled PVPP in the rat showed almost no absorption or gastrointestinal accumulation of orally dosed PVPP and no biliary excretion.

Six Sprague-Dawley rats received an oral dose of 250 mg
^{14}C-PVPP by gastric intubation.The PVPP used had been treated to remove polymers of less than 10,000 m.w.Approximately 0.128% of the ^{14}C-PVPP was excreted in the urine, most being excreted within the first 24 hours.80-99% of the ^{14}C was recovered in the feces in the 12-24 hour period.Less than 0.1% of the ^{14}C was recovered in the carcass, most being in the G.I. tract.There was no evidence of preferential binding of the ^{14}C to any organ or tissue.

Long-term clinical studies show that PVPP is unabsorbed in the gastrointestinal tract. No inherent pharmacological action, therefore, would be expected from PVPP excipients added as tablet disintegrants, particularly at common usage levels of 20-80 mg/tablet.

[1]Twenty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives, Geneva, WHO Tech. Rep. Ser. No. 696, 1983.
PVPP-2 11/16/94

4.3.B Specific phenolics – Quercetin, Myricetin and Kaempferol

One of the flavonoids in cranberry is quercetin. According to "Agency Response Letter GRAS Notice No. GRN 000030", there was concern over the amount of quercetin in ShanStar Cranberry since quercetin has been shown to be a carcinogenic substance. Analysis by a third party laboratory of a sample of ShanStar Cranberry, with the highest level of total phenolics within our specification range, showed levels of:

- Quercetin at ~ 2400 PPM or 2.4 mg/g
- Myrecetin at ~ 1650 PPM or 1.7 mg/g
- Kaempferol at ~ 34 PPM or 0.034 mg/g

All 3 of these compounds have been shown in some studies to be cancer inducing and in many studies to be anti-carcinogenic (References 1 - 91). As stated by the FDA the dose is the poison. Of the studies on quercetin quoted by the FDA in Response Letter No. GRN 000030, in those that showed evidence of carcongenicity the doses of quercetin are 40,000 PPM (US Dept. Of Health, 1992) and 1,000 PPM (Pamukcu et al, 1980). Using the same conservative estimate that the FDA used in response letter # GRN 000030, that ShanStar Cranberry is present in all foods and beverages, we estimate that a person who eats 1500 g of food per day and drinks 1500 ml of beverage per day would consume a total of 0.9 g of ShanStar Cranberry. This is with a maximum concentrations of ShanStar Cranberry of 0.5 mg/g of food and 0.1 mg/ml of beverage. This would result in a daily consumption of, 2.2 mg of quercetin, 1.5 mg of myricetin and 0.031 mg of kaempferol due to the addition of ShanStar Cranberry to ALL foods and beverages. We are ONLY planning to add this product to beverages, and to foods and beverages for special dietary use. Not all the food and beverage consumed on a daily basis will contain ShanStar. Based on our estimates in Section 4.8, the maximum daily dietary exposure to quercetin would be only 1.66 mg or 0.55 PPM.

Quercetin has been found in cranberry at levels of 100 to 250 ppm[1]. In a processed juice cocktail there is 2.4 PPM of quercetin, 1.4 PPM of myricetin and 0.14 PPM of kaempferol. In an international study on the estimated daily intake of quercetin from a varied and unrestricted diet, it was found that ~ 20 mg of quercetin is ingested in foods and beverages on a daily basis[2]. Other examples of quercetin content in foods and beverages are: ~ 30 PPM in black tea, and ~ 100 PPM in onions[3].

Quercetin is also a common additive to anti-oxidant formulations of dietary supplements. A list of a few products containing quercetin is found below.

Product	**Quercetin per tablet or capsule**	**Recommended number of tablets or capsules per day**
Solgar – Quercetin Complex	250 mg	2 per day
The Apothecary- Quercetin +	500 mg	1 or 2 per day
Health World- Quercetin	500 mg	2 to 3 per day
Kilo Sports- Quercetin	400 mg	3 to 5, or more per day
Country Life- Super 10 Anti-oxidants	50 mg	2

These quantities of quercetin far exceed that found in ShanStar Cranberry and the quantity that one would be exposed to when using our estimated dietary exposure as in Section 4.8.

[1] Duke, James A. 1992. Handbook of phytochemical constituents of GRAS herbs and other economic plants. Boca Raton, FL. CRC Press.
[2] Cancer Lett 1997 Mar 19;114(1-2):141-4., *Consumption of quercetin and kaempferol in free-living subjects eating a variety of diets.* ***de Vries JH, et al.***

[3] Am J.Clin Nutr 1998 Jul;68(1):60-5 Plasma concentrations and urinary excretion of the antioxidant flavonols quercetin and kaempferol as biomarkers for dietary intake. de Vries JH, et al.

References Specific for Quercetin, Myricetin and Kaempferol

1. Eur J Clin Nutr 2000 Feb;54(2):143-9 .

Prediction of dietary flavonol consumption from fasting plasma concentration or urinary excretion
Noroozi M et al Department Human Nutrition, University of Glasgow, Glasgow, Scotland, UK.

OBJECTIVES: to predict flavonols content of the habitual diets of free-living subjects from urine and plasma concentrations of flavonols. DESIGN: Ten type 2 diabetic patients (five male, five female), mean age 60 (s.e.m. 7) y and BMI 30.2 (s.e.m. 3.5) kg/m2 were treated in a random crossover design for a 2 week period on either a low flavonoid diet or on the same diet supplemented at one of two high flavonols levels (total 77.3 or 110.4 mg/day) provided by supplements of 1500 ml tea daily and 400 g fried white onion in olive oil with and without tomato ketchup and herbs. SETTING: Glasgow Royal Infirmary, University of Glasgow, Scotland. MAIN OUTCOME MEASURES: Fasting plasma concentration, urine concentration and 24 h excretion of quercetin, isorhamnetin, kaempferol and myricetin. RESULTS: Plasma flavonol concentration (r=0.750, P=0.001), 24 h urine concentration (r=0.847, P=0.001) and 24 h urine excretion (r=0.728, P=<0.001) were all highly significantly to dietary intake and gave similar estimates of intakes. Fasting plasma flavonols concentrations on habitual diets ranged from 0 to 43.7 ng/ml mean. Regression equations were constricted: total flavonols intake r=0.74, P<0.001 and quercetin intake r=0.744, P<0. 001. From these equations, flavonol intakes from habitual diets were estimated at 17-50, mean 35 mg/day. Of this, 91% was from quercetin. CONCLUSIONS: Dietary flavonols are absorbed and appear in plasma and urine as potential biomarkers in concentrations quantitatively to intake. Estimation of dietary intake from plasma or urine concentrations appears possible. Sponsorship: Rank Prize Funds and Rank Foundation of the Department of Human Nutrition; Ministry of Health and Medical Education, IR Iran.

2. Med Invest 1999 Aug;46(3-4):159-68

Dietary flavonoids as antioxidants in vivo: conjugated metabolites of (-)-epicatechin and quercetin participate in antioxidative defense in blood plasma.
Terao J, Department of Nutrition, University of Tokushima School of Medicine, Japan.

Flavonoids are present in mainly plant foods and have attracted much attention in relation to disease prevention. Their antioxidant activity at least partly accounts for their potential health effect, because oxidative stress leads to a variety of pathophysiological events. It is

essential to know the bioavailability of flavonoids involving intestinal absorption, metabolic conversion and urinary excretion, in order to evaluate their in vivo antioxidant activity after intake. Here (-)-epicatechin and quercetin were selected as typical flavanol- and flavonol-flavonoids present in vegetables, fruits and tea. Our rat study suggests that their metabolic conversion begins in the intestinal mucosa where the activity of uridine-5'-diphosphoglucuronosyltransferase (UGT) is at its highest. Both flavonoids accumulated mostly as glucuronide and sulfate conjugates in blood plasma after oral administration. No intact quercetin was found in the circulation. However, on the oral administration of these flavonoids, the antioxidative ability of rat plasma was enhanced indicating that conjugated metabolites participate in the antioxidant defense in blood plasma. Therefore, the intake of vegetables, fruits and tea rich in flavonoids may help to prevent oxidative damages in the blood.

3. J Natl Cancer Inst 2000 Jan 19;92(2):154-60

Intake of flavonoids and lung cancer.

Le Marchand L, Murphy SP, Hankin JH, Wilkens LR, Kolonel LN, Etiology Program, Cancer Research Center of Hawaii, University of Hawaii, Honolulu 96813, USA. loic@crch.hawaii.edu

BACKGROUND: To investigate the possible relationship between intake of flavonoids-powerful dietary antioxidants that may also inhibit P450 enzymes-and lung cancer risk, we conducted a population-based, case-control study in Hawaii. METHODS: An in-person interview assessed smoking history and usual intake of 242 food items for 582 patients with incident lung cancer and 582 age-, sex-, and ethnicity-matched control subjects. Subjects who donated a blood sample were genotyped for the P450 enzyme variant allele CYP1A1*2 by use of a polymerase chain reaction-based method. Logistic regression analysis was used to compute
odds ratios (ORs) and 95% confidence intervals (CIs). All P values are two-sided. RESULTS: After adjusting for smoking and intakes of saturated fat and beta-carotene, we found statistically significant inverse associations between lung cancer risk and the main food sources of the flavonoids quercetin (onions and apples) and naringin (white grapefruit). The lung cancer OR for the highest compared with the lowest quartile of intake was 0.5 (95% CI = 0.3-0.9) for onions (P for trend =.001) and 0.6 (95% CI = 0.4-1.0) for apples (P for trend =.03). The OR for the highest compared with the lowest tertile of intake for white grapefruit was 0.5 (95% CI = 0.2-0.9) (P for trend =.02). No association was found for important food sources of other flavonoids. Using published food-composition data for flavonoids, we found an inverse association between intake of quercetin and risk of lung cancer (P for trend =.07) that appears consistent with associations for its food sources. The effect of onions was particularly strong against squamous cell carcinoma (a cell type specifically associated with CYP1A1*2 in our study) and was modified by the CYP1A1 genotype, suggesting that CYP1A1 may play a

role in this association. CONCLUSION: If replicated, particularly in prospective studies, these findings would suggest that foods rich in certain flavonoids may protect against certain forms of lung cancer and that decreased bioactivation of carcinogens by inhibition of CYP1A1 should be explored as underlying mechanisms.

4. Urology 1999 Dec;54(6):960-3

Quercetin in men with category III chronic prostatitis: a preliminary prospective, double-blind, placebo-controlled trial.

Shoskes DA, Zeitlin SI, Shahed A, Rajfer J. Institute for Male Urology, Encino, California, USA.

OBJECTIVES: The National Institutes of Health (NIH) category III chronic prostatitis syndromes (nonbacterial chronic prostatitis and prostatodynia) are common disorders with few effective therapies. Bioflavonoids have recently been shown in an open-label study to improve the symptoms of these disorders in a significant proportion of men. The aim of this study was to confirm these findings in a prospective randomized, double-blind, placebo-controlled trial. METHODS: Thirty men with category IIIa and IIIb chronic pelvic pain syndrome were randomized in a double-blind fashion to receive either placebo or the bioflavonoid quercetin 500 mg twice daily for 1 month. The NIH chronic prostatitis symptom score was used to grade symptoms and the quality-of-life impact at the start and conclusion of the study. In a follow-up unblind, open-label study, 17 additional men received 1 month of a supplement containing quercetin, as well as bromelain and papain (Prosta-O), which enhance bioflavonoid absorption. RESULTS: Two patients in the placebo group refused to complete the study because of worsening symptoms, leaving 13 placebo and 15 bioflavonoid patients for evaluation in the blind study. Both the quercetin and placebo groups were similar in age, symptom duration, and initial symptom score. Patients taking placebo had a mean improvement in NIH symptom score from 20.2 to 18.8 (not significant), while those taking the bioflavonoid had a mean improvement from 21.0 to 13.1 ($P = 0.003$). Twenty percent of patients taking placebo and 67% of patients taking the bioflavonoid had an improvement of symptoms of at least 25%. In the 17 patients who received Prosta-Q in the open-label study, 82% had at least a 25% improvement in symptom score. CONCLUSIONS: Therapy with the bioflavonoid quercetin is well tolerated and provides significant symptomatic improvement in most men with chronic pelvic pain syndrome.

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5. Arch Biochem Biophys 1999 Dec 15;372(2):347-54

Inhibitory effect of quercetin metabolites and their derivatives on copper ion-induced lipid peroxidation in human low-density lipoprotein.

Yamamoto N, Moon JH, Tsushida T, Nagao A, Terao J, Takeda Food Products. Ltd, Itami, Hyogo, 664-0011, Japan.

To determine the antioxidant activity of dietary quercetin (3,3',4', 5,7-pentahydroxyflavone) in the blood circulation, we measured the inhibitory effect of quercetin metabolites and their derivatives on copper ion-induced lipid peroxidation of human low-density lipoprotein (LDL). Conjugated quercetin metabolites were prepared from the plasma of rat 1 h after oral administration of quercetin aglycone (40 micromol/rat). The rate of cholesteryl ester hydroperoxide (CE-OOH) accumulation and the rate of alpha-tocopherol consumption in mixtures of LDL solution (0.4 mg/ml) with equal volumes of this preparation were slower than the rates in mixtures of LDL with preparations from control rats. The concentrations of CE-OOH after 2 h oxidation in the mixtures of LDL with preparations of conjugated quercetin metabolites were significantly lower than those in the control preparation. It is therefore confirmed that conjugated quercetin metabolites have an inhibitory effect on copper ion-induced lipid peroxidation in human LDL. Quercetin 7-O-beta-glucopyranoside (Q7G) and rhamnetin (3,3',4', 5-tetrahydroxy-7-methoxyflavone) exerted strong inhibition and their effect continued even after complete consumption, similarly to quercetin aglycone. The effect of quercetin 3-O-beta-glucopyranoside (Q3G) did not continue after its complete consumption, indicating that the antioxidant mechanism of quercetin conjugates lacking a free hydroxyl group at the 3-position is different from that of the other quercetin conjugates. The result that 4'-O-beta-glucopyranoside (Q4'G) and isorhamnetin (3,4',5, 7-tetrahydroxy-3'-methoxyflavone) showed little inhibition implies that introduction of a conjugate group to the position of the dihydroxyl group in the B ring markedly decreases the inhibitory effect. The results of azo radical-induced lipid peroxidation of LDL and the measurement of free radical scavenging capacity using stable free radical, 1,1,-diphenyl-2-picrylhydrazyl, demonstrated that the o-dihydroxyl-structure in the B ring is required to exert maximum free radical scavenging activity. It is therefore likely that conjugation occurs at least partly in positions other than the B ring during the process of metabolic conversion so that the inhibitory effect of dietary quercetin is retained in blood plasma after absorption.

6. Food Chem Toxicol 1999 Sep-Oct;37(9-10):985-92

Phenolics: blocking agents for heterocyclic amine-induced carcinogenesis.

Hirose M, Takahashi S, Ogawa K, Futakuchi M, Shirai T, First Department of Pathology, Nagoya City University, Medical School, Nagoya, Japan.

Chemopreventive effects of synthetic and naturally occurring antioxidants on heterocyclic amine (HCA)-induced rat carcinogenesis and mechanisms of inhibition were assessed. In a medium-term liver bioassay, combined treatment with 0.03% 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx) and synthetic antioxidants such as 1-O-hexyl-2,3,5-trimethylhydroquinone (HTHQ), butylated hydroxyanisole (BHA), butylated hydroxutoluene (BHT), tert-butylhydroquinone (TBHQ) or propyl gallate, each at a dose of 0.25%, inhibited development of preneoplastic glutathione S-transferase placental form (GST-P) positive foci as compared with MeIQx alone, after initiation with diethylnitrosamine (DEN). Of these antioxidants, HTHQ showed the greatest activity. 8-Hydroxydeoxyguanosine (8-OHdG), a marker for DNA damage induced by active oxygen species, and malonedialdehyde and 4-hydroxynonenal levels were not largely influenced by the treatment with MeIQx or antioxidants, either alone or in combination. In the same medium-term liver bioassay, effects of some naturally occurring antioxidants, such as green tea catechins (GTC), hesperidin, chlorogenic acid, quercetin, rutin, curcumin, daidzin, ferulic acid and genistein were also examined. Of these antioxidants, only GTC tended to inhibit GST-P positive foci development, while quercetin, rutin, curcumin, daidzin, ferulic acid and genistein all exerted significant enhancing effects. Examination of HTHQ influence in a medium term liver bioassay with HCA Glu-P-1, in which the experimental period was extended for up to 26 weeks, also demonstrated a significant decrease in the incidence of liver tumours to 40% in the group treated with 0.5% HTHQ and 0.03% 2-amino-6-methyldipyrido[1,2-a:3',2'-d]imidazole (Glu-P-1) as compared with the Glu-P-1 alone value of 89%. Effects of HTHQ on colon carcinogenesis induced by 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) were evaluated in a two-stage colon carcinogenesis model using 1,2-dimethylhydrazine (DMH) as an initiator. At week 36, the multiplicity of colon tumours induced by 0.02% PhIP after DMH initiation (9.1+/-6.2/rat) was dose-dependently decreased by the combined treatment with 0.5% HTHQ (3.6+/-1.8, $P < 0.001$) and 0.125% HTHQ (6.2+/-3.2, not significant). Similarly, the incidence of mammary carcinomas in female F344 rats induced by oral administration of 0.02% PhIP (40%) for 52 weeks was significantly decreased by simultaneous treatment with 0.5% HTHQ (5%). Alpha-tocopherol and chlorophyllin only reduced the multiplicity of carcinomas. Analysis of the influence of HTHQ on metabolic activation of Glu-P-1 or PhIP after incubation with rat S9 mixture and NADPH by HPLC, revealed that each major metabolite was strongly reduced by the addition of HTHQ. Immunohistochemically detected PhIP-DNA adduct positive nuclei

in the colon induced by continuous oral treatment with 0.02% PhIP for 2 weeks decreased by the combined treatment with 0.5 or 0.125% HTHQ. These results indicate that synthetic antioxidant HTHQ is a very strong chemopreventor of heterocyclic amine (HCA)-induced carcinogenesis and that depressed metabolic activation rather than antioxidant activity is responsible for the observed effect.

7. Food Chem Toxicol 1999 Sep-Oct;37(9-10):937-42

Dietary flavonoids: intake, health effects and bioavailability.

Hollman PC, Katan MB , Wageningen Centre for Food Sciences, State Institute for Quality Control of Agricultural Products (RIKILT-DLO), The Netherlands.

Flavonoids are polyphenolic compounds that occur ubiquitously in foods of plant origin. Over 4000 different flavonoids have been described. They may have beneficial health effects because of their antioxidant properties and their inhibitory role in various stages of tumour development in animal studies. An estimation of the total flavonoid intake is difficult, because only limited data on food contents are available. It is estimated that humans ingest a few hundreds of milligram per day. The average intake of the subclasses of flavonols and flavones in The Netherlands was 23 mg/day. The intake of flavonols and flavones was inversely associated with subsequent coronary heart disease in most but not all prospective epidemiological studies. A protective effect of flavonols on cancer was found in only one prospective study. Flavonoids present in foods were considered non-absorbable because they are bound to sugars as beta-glycosides. However, we found that human absorption of the quercetin glycosides from onions (52%) is far better than that of the pure aglycone (24%). Flavonol glycosides might contribute to the antioxidant defences of blood. Dietary flavonols and flavones probably do not explain the cancer-protective effect of vegetables and fruits; a protective effect against cardiovascular disease is not conclusive.

8. Cancer Lett 1999 Sep 1;143(2):173-8

Chemoprevention of heterocyclic amine-induced carcinogenesis by phenolic compounds in rats.

Hirose M, Takahashi S, Ogawa K, Futakuchi M, Shirai T, Shibutani M, Uneyama C, Toyoda K, Iwata H , Division of Pathology, Biological Safety Research Center, National Institute of Health Sciences, Tokyo, Japan. m-hirose@nihs.go.jp

Chemopreventive effects of synthetic and naturally occurring antioxidants on heterocyclic amine (HCA)-induced rat carcinogenesis and mechanisms of inhibition were assessed. In a medium-term liver bioassay, combined treatment with 0.03% 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx) and synthetic antioxidants such as 1-O-hexyl-2,3,5-trimethylhydroquinone (HTHQ), BHA, BHT, tert-butylhydroquinone (TBHQ) and propyl gallate, each at a dose of 0.25%, and troglitazone at doses 0.5 and 0.1%, potently inhibited development of glutathione S-transferase placental form (GST-P) positive foci as compared with MeIQx alone values. Of these antioxidants, HTHQ showed the greatest activity. Green tea catechins tended to inhibit GST-P positive foci development, while quercetin, rutin, curcumin, daidzin, ferulic acid and genistin all exerted significant enhancing effects. HTHQ also inhibited 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP)-induced colon carcinogenesis in a two stage colon carcinogenesis model using 1,2-dimethylhydrazine (DMH) as an initiator. Immunohistochemically detected PhIP-DNA adduct positive nuclei in the colon induced by continuous oral treatment with 0.02% PhIP for 2 weeks decreased by the combined treatment with 0.5 or 0.125% HTHQ. Methoxyresorfin O-demethylase activity in rat liver microsomes in vitro was clearly inhibited by the addition of HTHQ, BHA, BHT, TBHQ or propyl gallate, with particularly strong inhibition being observed in HTHQ. However, the CYP1A2 level in rat liver increased after oral treatment with HTHQ for 2 weeks. These results indicate that synthetic antioxidants, HTHQ in particular, is a very strong chemopreventor of HCA-induced carcinogenesis. It is suggested that depression of metabolic activation rather than antioxidant activity is responsible for the observed effect. However, other mechanisms, including the effects on phase II enzymes cannot be ruled out.

9. Food Chem Toxicol 1999 Apr;37(4):313-8

Prevention of N-nitrosodiethylamine-induced lung tumorigenesis by ellagic acid and quercetin in mice.

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The polyphenolic antioxidants, consumed as an integral part of vegetables, fruits and beverages, are suggested as possessing anticarcinogenic properties. In the present study we have looked into the anticarcinogenic potential of plant polyphenols ellagic acid (EA) and quercetin against N-nitrosodiethylamine-induced lung tumorigenesis in mice. Ellagic acid was able to significantly reduce tumour incidence

to 20% from the control value of 72.2%. Similarly, tumour burden was also decreased, although not significantly, from 3.15 to 2.5. Quercetin (QR) caused the tumour incidence to decrease from 76.4% to 44.4% when fed until the third dose of carcinogen. Both of the polyphenols suppressed the tumour incidence mainly by acting at the initiation phase of the carcinogenesis, since continuing the feeding of polyphenols until the termination of the experiment did not cause any apparent change in tumour incidence or tumour burden. Besides this, ellagic acid was found to be a better chemopreventor than quercetin. In order to search for their mechanism of action, the effect of feeding of these compounds on reduced glutathione (GSH), an important endogenous antioxidant, and on lipid peroxidation was investigated. Both ellagic acid and QR caused a significant increase in GSH and decrease in NADPH- and ascorbate-dependent lipid peroxidation. Ellagic acid was found to be more effective in decreasing the lipid peroxidation and increasing the GSH. This may be one of the reasons for its observed better anticarcinogenic property as compared to quercetin.

10. Int J Clin Pharmacol Ther 1999 May;37(5):219-33

Pharmacokinetics and bioavailability of the flavonol quercetin in humans.

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Flavonoids are plant polyphenolic compounds present in the daily diet. Latest epidemiological studies point to a crucial role of the flavonol quercetin in the prevention of cardiovascular diseases. It is assumed that this protective effect derives from the antioxidative capacity which quercetin shows in in vitro experiments. The antiproliferative and antimutagenic activities in vitro have made it a candidate for clinical trials in cancer therapy. Quercetin is also regarded as a putative active compound in various phytopharmaceuticals. However, in vivo data on the disposition, absorption, bioavailability, and metabolism of quercetin after intravenous and oral administration in humans are scarce and contradictory. The pharmacokinetic parameters following intravenous injection were determined in two studies. The elimination half-life was reported to be 2.4 h and 0.7 h, the volume of distribution at steady-state was 92.6 l and 6.2 l, and total body clearance was 34.6 lxh(-1) and 28.1 lxh(-1), respectively. Absorption after oral administration ranged from 0 to over 50% of the dose. These inconsistencies can partly be attributed to a lack of highly sensitive

and specific assay methodology. The data available so far are insufficient to clarify whether or not quercetin can be held responsible for any protective or curative effect observed after oral intake.

11. Eur J Nutr 1999 Feb;38(1):28-34

Dietary flavonoids protect human colonocyte DNA from oxidative attack in vitro.

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BACKGROUND AIMS: Epidemiological studies suggest that antioxidant polyphenols in the human diet may protect against diseases such as cancer. In this study we investigated the cytoprotective potential of the flavonoids, quercetin, myricetin, kaempferol and rutin against oxidative DNA damage in human colonocytes in vitro. METHODS: Caco-2 cells, which display specialised enterocyte/colonocyte cell functions, were used as an in vitro model for human colonocytes. Hydrogen peroxide was employed as the oxidant. DNA damage (strand breakage, oxidised purines and oxidised pyrimidines) was determined using the alkaline single cell gel electrophoresis or comet assay. Cell growth and viability were measured. RESULTS: Hydrogen peroxide caused a dose-dependent increase in DNA strand breakage in human colonocytes, presumably via oxygen free radical generation. Quercetin and myricetin protected Caco-2 cells against oxidative attack. In addition, quercetin decreased hydrogen peroxide-mediated inhibition of growth. Neither rutin nor kaempferol was effective. However, quercetin, while inhibiting DNA strand breakage, did not alter the levels of oxidised bases following peroxide treatment. The antifungal agent ketoconazole, prevented quercetin cytoprotection in Caco-2 cells, indicating that P450-mediated metabolism may alter the efficacy of the flavonoids against oxidative DNA damage. CONCLUSION: Flavonoids, particularly quercetin, the most abundant flavonoid in the human diet, are likely to be important in defending human colonocytes from oxidative attack.

12. Cancer Causes Control 1999 Feb;10(1):71-5

Intake of specific carotenoids and flavonoids and the risk of gastric cancer in Spain.

Garcia-Closas R, Gonzalez CA, Agudo A, Riboli E
Research Unit of the University Hospital of Canarias, Tenerife, Spain.

OBJECTIVES: To investigate the relationship between gastric cancer and the intake of specific carotenoids (alpha-carotene, beta-carotene, lutein, and lycopene) and flavonoids (quercetin, kaempferol, myricetin, and luteolin) using new data on their concentration in foods. METHODS: Case-control study carried out in Spain that included 354 cases of gastric cancer and 354 controls, matched by age, gender, area of residence and hospital. Usual food intake was assessed using a dietary history questionnaire. RESULTS: In a multivariate model adjusted for several dietary factors, no association was found between intake of any of the studied carotenoids and the risk of gastric cancer. The adjusted OR of gastric cancer for the highest quartile of total flavonoid intake versus the lowest quartile was 0.44 (95 percent confidence interval [CI] = 0.25-0.78; P for trend = 0.003). Kaempferol intake was found to be protective (OR = 0.48; CI = 0.26-0.88; P for trend = 0.04) comparing the highest versus the lowest quartile of intake. A trend toward lower risk of stomach cancer with higher intake of quercetin was also found. CONCLUSIONS: The results of this study support the hypothesis that the well-established protective effect of fruit and vegetables against gastric cancer could, in part, be due to the presence of flavonoids.

13. Mutat Res 1999 Apr 26;441(1):1-9

Antimutagenic effects of natural phenolic compounds in beans.

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Polyphenols in fruits, vegetables (e.g., flavonols like quercetin) and tea (e.g., catechins such as epigallocatechin gallate) are good antioxidants with antimutagenic and anticarcinogenic properties. In the present study, the Salmonella typhimurium tester strain YG1024 was used in the plate-incorporation test to examine the antimutagenic effect of phenolic compounds, extracted from common beans (Phaseolus vulgaris), on 1-NP and B[a]P mutagenicity. Dose-response curves for 1-NP and B[a]P were obtained; the number of net revertants/plate at the peak mutagenic dosage were 880 for 1-NP and 490 for B[a]P. For the antimutagenicity studies doses of 0.1 microg/plate and 2 microg/plate for 1-NP and B[a]P, respectively, were chosen. We obtained a dose-response curve of ellagic

acid (EA) against B[a]P and 1-NP mutagenicity. To test the bean extract, a dose of 300 microg/plate of EA was chosen as the antimutagenic control. The EA and bean extracts were not toxic to the bacteria at the concentrations tested. The inhibitory effects of the bean extracts and EA against B[a]P mutagenicity were dose-dependent. The percentages of inhibition produced against B[a]P (2 microg/plate) using 300 microg/plate of EA and for the extracts 500 microg equivalent catechin/plate were 82%, 83%, 81% and 83% for EA, water extract, water/methanol extract and methanol extract, respectively. However, for 1-NP mutagenicity, only the methanolic extract from beans showed an inhibitory effect. These results suggest that common beans, as other legumes, can function as health-promoting foods.

14. Anticancer Drugs 1999 Feb;10(2):187-93

Modulating effect of resveratrol and quercetin on oral cancer cell growth and proliferation.

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Resveratrol and quercetin are polyphenols which have been detected in significant amounts in green vegetables, citrus fruits and red grape wines. Beneficial effects attributed to these compounds include anti-inflammatory, antiviral and antitumor properties. The effect of resveratrol and quercetin on growth of human oral cancer cells is unknown. Resveratrol and quercetin, in concentrations of 1 to 100 microM, were incubated in triplicates with human oral squamous carcinoma cells SCC-25 in DMEM-HAM's F-12 supplemented with fetal calf serum and antibiotics in an atmosphere of 5% CO2 in air at 37 degrees C for 72 h. Cell growth was determined by counting the number of viable cells with a hemocytometer. Cell proliferation was measured by means of incorporation of [3H]thymidine in nuclear DNA. Resveratrol at 10 and 100 microM induced significant dose-dependent inhibition in cell growth as well as in DNA synthesis. Quercetin exhibited a biphasic effect, stimulation at 1 and 10 microM, and minimal inhibition at 100 microM in cell growth and DNA synthesis. Combining 50 microM of resveratrol with 10, 25 and 50 microM of quercetin resulted in a gradual and significant increase in the inhibitory effect of quercetin on cell growth and DNA synthesis. We conclude that resveratrol or a combination of resveratrol and quercetin, in concentrations equivalent to that present in red wines, are effective inhibitors of oral squamous carcinoma cell (SCC-25) growth and proliferation, and warrant further investigation as cancer chemopreventive agents.

15. Free Radic Res 1999 Feb;30(2):153-62

The polyphenolic content of fruit and vegetables and their antioxidant activities. What does a serving constitute?

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Analysis of the major flavone, flavonol, anthocyanidin and hydroxycinnamic acid constituents (and their glycosides) of onion, tomato, egg plant and apple has been undertaken and the antioxidant activities of the phenolic extracts determined. The major phenolic antioxidant components of egg plant are chlorogenic acid in the flesh and a delphinidin conjugate in the skin. In the case of apple, the major phenolic antioxidants detected are chlorogenic acid, procyanidins/catechin compounds, rutin and phloridzin. Quercetin glycosides are well-known to be the major phenolic components of onion. Assessment of the antioxidant activities of a serving of 100g fresh weight fruit, vegetable and comparison with previously reported findings for 150 ml beverage (500 ml portion in the case of beer), expressed in micromol Trolox equivalents show that the antioxidant activities of 1 glass (150 ml) red wine equivalent to 12 glasses white wine equivalent to 2 cups of tea equivalent to 4 apples equivalent to 5 portions of onion equivalent to 5.5 portions egg plant equivalent to 3.5 glasses of blackcurrant juice equivalent to 3.5 (500 ml) glasses of beer equivalent to 7 glasses of orange juice equivalent to 20 glasses of apple juice (long life).

16. Eur J Clin Nutr 1999 Feb;53(2):92-6

Absorption and antioxidant effects of quercetin from onions, in man.(Human study)

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OBJECTIVE: To investigate the in vivo effects of quercetin following the ingestion of fried onions. DESIGN: Five healthy volunteers, three males and two females aged between 25 and 39 y, ingested 225 g of fried onions after an overnight fast and peripheral venous blood was collected 0, 2, 4, 24 and 48 h after consumption. Quercetin in the plasma, total antioxidant capacity and susceptibility of low density lipoproteins (LDL) to oxidation were measured. RESULTS: Following the onion meal, quercetin levels increased from baseline values (28.4 +/- 1.9 ng/ml) to peak after 2 h (248.4 +/- 103.9 ng/ml), decreasing to baseline again after 24 h ($P > 0.05$). This was accompanied by an increase in the total

antioxidant activity of the plasma from baseline (1.70 +/- 0.04 mmol/l trolox equivalents) to 1.75 +/- 0.10 mmol/l trolox equivalents after 2 h and 1.76 +/- 0.08 mmol/l trolox equivalents after 4 h. There was no significant change in the susceptibility of the plasma or the isolated LDL to oxidation over the 48 h period after consumption of the fried onions. In view of these negative findings, we isolated LDL and other lipoproteins from plasma at each time point. Quercetin was not detected in either LDL or VLDL, but was present in the HDL fraction, although this fraction also contains other proteins including albumin.
CONCLUSIONS: Quercetin can be absorbed in humans from dietary sources to high enough concentrations to increase the overall antioxidant activity of the plasma. Quercetin, however, has a strong affinity for protein and provides no direct protective effect during LDL oxidation.

17. Nutr Cancer 1998;32(3):154-8

Intake of specific carotenoids and flavonoids and the risk of lung cancer in women in Barcelona, Spain.

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Newly available data of a case-control study of lung cancer in women in Spain were analyzed to assess the relationship with the intake of specific carotenoids (alpha-carotene, beta-carotene, lutein, and lycopene) and flavonoids (quercetin, kaempferol, myricetin, and luteolin). The study included 103 cases and 206 hospital controls, matched by age and residence. Usual food intake was estimated through a food-frequency questionnaire. With adjustment for smoking habit and vitamin E, vitamin C, and total flavonoid intake, no association was found for the intake of alpha-carotene, beta-carotene, or lutein. The odds ratio (95% confidence interval) for women in the highest tertile of lycopene intake with respect to the lowest was 0.56 (0.26-1.24), with p for trend = 0.15. A nonsignificant association was observed for the highest vs. lowest tertile intake of kaempferol (odds ratio = 0.51, 95% confidence interval = 0.22-1.17), with p for trend = 0.10, after adjustment for smoking and vitamin E, vitamin C, and total carotenoid intake. No protective effect was observed for quercetin or luteolin or for total flavonoid intake.

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18. Am J Clin Nutr 1999 Jan;69(1):87-94

Effect of fruit juice intake on urinary quercetin excretion and biomarkers of antioxidative status.

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BACKGROUND: Epidemiologic studies suggest that foods rich in flavonoids might reduce the risk of cardiovascular disease. OBJECTIVE: Our objective was to investigate the effect of intake of flavonoid-containing black currant and apple juice on urinary excretion of quercetin and on markers of oxidative status. DESIGN: This was a crossover study with 3 doses of juice (750, 1000, and 1500 mL) consumed for 1 wk by 4 women and 1 man corresponding to an intake of 4.8, 6.4, and 9.6 mg quercetin/d. RESULTS: Urinary excretion of quercetin increased significantly with dose and with time. The fraction excreted in urine was 0.29-0.47%. Plasma quercetin did not change with juice intervention. Plasma ascorbate increased during intervention because of
the ascorbate in the juice. Total plasma malondialdehyde decreased with time during the 1500-mL juice intervention, indicating reduced lipid oxidation in plasma. Plasma 2-amino-adipic semialdehyde residues increased with time and dose, indicating a prooxidant effect of the juice, whereas erythrocyte 2-aminoadipic semialdehyde and gamma-glutamyl
semialdehyde concentrations, Trolox-equivalent antioxidant capacity, and ferric reducing ability of plasma did not change. Glutathione peroxidase activity increased significantly with juice dose. CONCLUSIONS: Urinary excretion of quercetin seemed to be a small but constant function of quercetin intake. Short-term, high intake of black currant and apple
juices had a prooxidant effect on plasma proteins and increased glutathione peroxidase activity, whereas lipid oxidation in plasma seemed to decrease. These effects might be due to several components of the juice and cannot be attributed solely to its quercetin content.

19. Jpn J Pharmacol 1998 Dec;78(4):435-41

Anti-ulcer effects of antioxidants, quercetin, alpha-tocopherol, nifedipine and tetracycline in rats.

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When free radical-scavenging activities of quercetin, alpha-tocopherol, nifedipine and tetracycline were measured by an electron spin resonance technique, all test compounds (10(-5) to 10(-3) M) scavenged both superoxide anions and hydroxyl radicals. The oral administration of quercetin (50 and 100 mg/kg), alpha-tocopherol (8 and 16 mg/kg), nifedipine (20 and 40 mg/kg) or tetracycline (10 and 20 mg/kg) markedly prevented the HCl plus ethanol-induced gastric mucosal injury and the increase in the content of thiobarbituric acid-reactive substances in the injured mucosa in rats. In addition, quercetin (25, 50 and 100 mg/kg), alpha-tocopherol (4, 8 and 16 mg/kg), nifedipine (10, 20 and 40 mg/kg) and tetracycline (5, 10 and 20 mg/kg), given orally, twice daily for 14 consecutive days from the day after acetic acid injection, dose-dependently promoted the ulcer healing and inhibited the increase in the content of thiobarbituric acid-reactive substances in the ulcerated mucosa. These results indicate that quercetin, alpha-tocopherol, nifedipine and tetracycline possess gastric cytoprotective and gastric ulcer healing-promoting actions. In addition, the free radical-scavenging properties of these compounds may be partly to their anti-ulcer effects.

20. Clin Cancer Res 1996 Apr;2(4):659-68

Phase I clinical trial of the flavonoid quercetin: pharmacokinetics and evidence for in vivo tyrosine kinase inhibition.

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We have performed a Phase I clinical trial with the naturally occurring flavonoid quercetin (3,3',4',5,7-pentahydroxyflavone). Quercetin has antiproliferative activity in vitro and is known to inhibit signal transduction targets including tyrosine kinases, protein kinase C, and phosphatidyl inositol-3 kinase. Quercetin was administered by short i.v. infusion at escalating doses initially at 3-week intervals. The first dose level was 60 mg/m2; at the 10th dose level of 1700 mg/m2, dose-limiting nephrotoxicity was encountered, but no myelosuppression. At the preceding dose level of 1400 mg/m2, five patients were treated at 3-week intervals, and another eight patients were treated on a once-weekly schedule; overall, 2 of 10 evaluable patients had renal

toxicity, 1 at grade 2 and 1 at grade 4. We therefore treated other patients at 945 mg/m2 (eight at 3-week intervals and six at weekly intervals); 3 of 14 patients had clinically significant renal toxicity, 2 patients with grade 2 and 1 patient with grade 3. Patients treated on the weekly schedule did not have cumulative renal impairment but did have a fall in the glomerular filtration rate of 19 +/- 8% in the 24 h after drug administration. We recommend 1400 mg/m2 as the bolus dose, which may be given either in 3-week or weekly intervals, for Phase II trials. Quercetin pharmacokinetics were described by a first-order two-compartment model with a median t(1/2)alpha of 6 min and median t(1/2)beta of 43 min. The median estimated clearance was 0.28 liter/min/m2, and median volume of distribution at steady state was 3.7 liter/m2. In 9 of 11 patients, lymphocyte protein tyrosine phosphorylation was inhibited following administration of quercetin at 1 h, which persisted to 16 h. In one patient with ovarian cancer refractory to cisplatin, following two courses of quercetin (420 mg/m2), the CA 125 had fallen from 295 to 55 units/ml, and in another patient with hepatoma, the serum alpha-fetoprotein fell. In conclusion, quercetin can be safely administered by i.v. bolus at a dose injection. The plasma levels achieved inhibited lymphocyte tyrosine kinase activity, and evidence of antitumor activity was seen.

21. Free Radic Res 1998 Sep;29(3):257-69

Absorption and excretion of conjugated flavonols, including quercetin-4'-O-beta-glucoside and isorhamnetin-4'-O-beta-glucoside by human volunteers after the consumption of onions.

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Flavonols are polyphenols found ubiquitously in plants and plant-products. Flavonols, particularly quercetin, are potent antioxidants in vitro and their intake has been associated inversely with the incidence of coronary heart disease. The aim of this study was to investigate the accumulation in plasma and excretion in urine of flavonol glucosides following ingestion of lightly fried onions. Five healthy volunteers followed a low-flavonoid diet for 3 days. On day 4, after an overnight fast, subjects were given 300 g of lightly fried yellow onions which contain conjugates of quercetin and isorhamnetin, including quercetin-3,4 '-diO-beta-glucoside, isorhamnetin-4'-O-beta-glucoside and quercetin-4'-O-beta-glucoside. Blood collection was carried out at 0 min, 0.5, 1.0, 1.5, 2, 3, 4, 5 and 24h after the supplement. In addition, subjects collected all their urine for 24h

following the onion supplement. Isorhamnetin-4'-O-beta-glucoside and quercetin-4 '-O-beta-glucoside accumulated in plasma with maximum levels, defined as proportion of intake, of 10.7+/-2.6% and 0.13+/-0.03% respectively. The time of the quercetin-4'glucoside peak plasma concentration was 1.3+/-0.2 h after the ingestion of onions while a value of 1.8+/-0.7 h was obtained for isorhamnetin-4'-glucoside. Excretion in urine, as a proportion of intake, was 17.4+/-8.3% for isorhamnetin-4'-O-beta-glucoside and 0.2+/-0.1% for quercetin-4'-O-beta-glucoside. Possible reasons for the accumulation and excretion of isorhamnetin-4'-glucoside in proportionally much higher amounts than quercetin-4'-glucoside are discussed. It is concluded that flavonols are absorbed into the bloodstream as glucosides and minor structural differences affect markedly both the level of accumulation and the extent to which the conjugates are excreted.

22. Transplantation 1998 Jul 27;66(2):147-52

Effect of bioflavonoids quercetin and curcumin on ischemic renal injury: a new class of renoprotective agents.

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BACKGROUND: Nonimmune renal injury plays an important role in acute and chronic rejection by triggering an injury response through cytokine and chemokine release. Bioflavonoids are agents with potential immunosuppressive and renoprotective properties. We studied the effects of quercetin and curcumin, two bioflavonoids, on ischemia-reperfusion in the rat. METHODS: Rats underwent 30 min of left renal pedicle occlusion with simultaneous right nephrectomy and were pretreated with quercetin or curcumin. Serial serum creatinine was measured, and renal expression of the chemokines regulated upon activation, normal T-cell expressed and secreted (RANTES), monocyte chemoattractant protein-1 (MCP-1), and allograft inflammatory factor (AIF) was quantified by polymerase chain reaction. RESULTS: Pretreatment with quercetin or curcumin resulted in preservation of histological integrity, with a decrease in tubular damage and interstitial inflammation. On day 2 after ischemia-reperfusion, quercetin pretreatment decreased the mean serum creatinine level from 6.5+/-1.4 to 3.3+/-0.5 mg/dl (P=0.06). On day 7, the creatinine level for control animals was 7.5+/-1.5 mg/dl, which was significantly decreased by pretreatment with quercetin, curcumin, or both together (creatinine levels: 1.6+/-1.3, 1.8+/-0.2, and 2.0+/-0.4 mg/dl, respectively; all P<0.05 vs. untreated). By semiquantitative polymerase chain reaction, RANTES, MCP-1, and AIF were detected at high levels in kidneys on day 2 but not in normal kidneys. Pretreatment with

quercetin or curcumin strongly attenuated this expression. CONCLUSION: Quercetin and curcumin reduce ischemia-reperfusion injury and its inflammatory sequelae. The bioflavonoids hold promise as agents that can reduce immune and nonimmune renal injury, the key risk factors in chronic graft loss.

23. Mutat Res 1998 Jun 18;402(1-2):279-88

Prevention by inhibitors of arachidonic acid cascade of liver carcinogenesis, cirrhosis and oxidative DNA damage caused by a choline-deficient, L-amino acid-defined diet in rats.

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Effects of inhibitors of arachidonic acid (AA) cascade on the development of fatty liver, cirrhosis, glutathione S-transferase placental form (GST-P)-positive preneoplastic nodules, neoplastic nodules and generation of 8-hydroxydeoxyguanosine (8-OHdG), caused by a
choline-deficient, L-amino acid-defined (CDAA) diet, were examined in Fischer 344 male rats by feeding CDAA diet supplemented with the inhibitors for 12 and 30 weeks. None of the inhibitors affected fatty liver. Among cyclooxygenase (COX) inhibitors, an irreversibly acting acetylsalicylic acid and a long-acting piroxicam, and to a much lesser extent the short-acting ibuprofen but not indomethacin, inhibited the development of cirrhosis, GST-P-positive and neoplastic nodules and generation of 8-OHdG. A phospholipase A2 inhibitor p-bromophenacylbromide (BPB) also exerted similar but lesser extent of inhibitory effects. Lipoxygenase inhibitors quercetin and nordihydroguiaretic acid inhibited GST-P-positive nodules but not cirrhosis or 8-OHdG. Present results suggest that perturbed AA cascade, particularly augmented COX pathway, might play key roles in the
causation of liver lesions in the CDAA diet model.

24. Mutat Res 1998 Jun 18;402(1-2):219-24

Antimutagenic dietary phenolics as antigenotoxic substances in urothelium of smokers.

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Human urine is known to contain substances that strongly inhibit

bacterial mutagenicity of aromatic and heterocyclic amines in vitro. The biological relevance of these anti-mutagens was examined by comparing levels of tobacco-DNA adducts in exfoliated urothelial cells from smokers with the anti-mutagenic activity in corresponding 24-h urine samples. An inverse relationship was found between the inhibition of PhIP-mutagenicity by urine extracts in vitro and two DNA adduct measurements: the level of the putatively identified ABP-dG adduct and the total level of all tobacco-smoke-carcinogen adducts including those probably derived from PhIP. These substances appear to be dietary phenolics and/or their metabolites because (i) the anti-mutagenic activity of urine extracts (n=18) was linearly to their content in phenolics; (ii) the concentration ranges of these substances in urine extracts were similar to those of various plant phenols (e.g., quercetin, isorhamnetin) for which an inhibitory effect on the liver S9-mediated mutagenicity of PhIP was obtained; (iii) treatment of urines with beta-glucuronidase and arylsulfatase enhanced both anti-mutagenicity and the levels of phenolics in urinary extracts; (iv) urinary extracts inhibited non-competitively the liver S9-mediated mutagenicity of PhIP as did quercetin, used as a model phenolics. Onion, lettuce, apples and red wine are important sources of dietary flavonoids which are probably responsible for the anti-mutagenicity associated with foods and beverages. After HPLC fractionation of urinary extracts, the distribution profile of anti-mutagenic activity corresponded roughly to that of onion and wine extract combined. Overall, our study strongly suggests that smokers ingesting dietary phenolics, probably flavonoids, are partially protected against the harmful effects by tobacco carcinogens within their bladder mucosal cells.

25. Microbios 1998;93(374):43-54

Comparative antibacterial and antifungal effects of some phenolic compounds.

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The antimicrobial potential of eight phenolic compounds isolated from olive cake was tested against the growth of Escherichia coli, Klebsiella pneumoniae, Bacillus cereus, Aspergillus flavus and Aspergillus parasiticus. The phenolic compounds included p-hydroxy benzoic, vanillic, caffeic, protocatechuic, syringic, and p-coumaric acids,

oleuropein and quercetin. Caffeic and protocatechuic acids (0.3 mg/ml) inhibited the growth of E. coli and K. pneumoniae. The same compounds apart from syringic acid (0.5 mg/ml) completely inhibited the growth of B. cereus. Oleuropein, and p-hydroxy benzoic, vanillic and p-coumaric acids (0.4 mg/ml) completely inhibited the growth of E. coli, K. pneumoniae and B. cereus. Vanillic and caffeic acids (0.2 mg/ml) completely inhibited the growth and aflatoxin production by both A. flavus and A. parasiticus, whereas the complete inhibition of the moulds was attained with 0.3 mg/ml p-hydroxy benzoic, protocatechuic, syringic, and p-coumaric acids and quercetin.

26. Am J Clin Nutr 1998 Jul;68(1):60-5

Plasma concentrations and urinary excretion of the antioxidant flavonols quercetin and kaempferol as biomarkers for dietary intake.

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Flavonols are antioxidants that may reduce the risk of heart disease. Two major flavonols in the diet are quercetin and kaempferol, and their main sources in The Netherlands are tea and onions. We investigated whether plasma concentrations and urinary excretion of quercetin and kaempferol in humans could be used as biomarkers of intake. We provided 15 subjects with strong black tea (1600 mL/d) or fried onions (129 g/d) for 3 d each in random order separated by a 4-d washout period. The tea provided 49 mg quercetin and 27 mg kaempferol daily and the onions provided 13 mg quercetin and no kaempferol. Flavonols from both foods were clearly absorbed. However, the excretion of unmodified quercetin was 0.5% of intake after tea and 1.1% after onions. Thus, the absorption of quercetin from tea was half of that from onions. The onion treatment was repeated 7-14 d later to estimate within-subject CVs as a measure of reproducibility when the same treatment is given twice. CVs for quercetin were 30% in plasma and 42% in urine. The magnitude of these variations relative to actual variations of approximately 60% between free-living subjects indicates that concentrations of quercetin in plasma and urine are applicable as biomarkers of its intake. We conclude that flavonols in plasma and urine reflect short-term flavonol intake and that they could be used as biomarkers to distinguish between high

and low flavonol consumption in epidemiologic studies.

27. JAMA 1998 May 13;279(18):1437-8

New developments in family medicine.

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28. Altern Med Rev 1998 Apr;3(2):140-3

Quercetin.

29. Immunopharmacol Immunotoxicol 1998 Feb;20(1):173-90

Dietary quercetin, immune functions and colonic carcinogenesis in rats.

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Rats fed 100 mg/kg quercetin (QUE) daily for 7 weeks had significantly enhanced natural killer cell activity compared to their vehicle (VEH)-fed control. In contrast, rats fed 100 mg/kg QUE and treated with the colon carcinogen, azoxymethane had significantly reduced natural killer cell activity compared to their VEH-fed azoxymethane-treated control. There was no significant difference in natural killer cell activity between the two control groups. Antibody production and delayed-type hypersensitivity were not altered by QUE feeding in any treatment group. In vitro exposure of splenic natural killer cells to 1mM QUE significantly decreased natural killer cell cytotoxicity. Lower QUE concentrations produced a non-significant reduction in natural killer cell activity that was restored to control values at 1 x 10(-13)M QUE. The distribution, multiplicity and total number of colonic preneoplastic lesions, aberrant crypt foci, was not significantly different in the QUE-fed azoxymethane-treated rats when compared to azoxymethane-treated vehicle-fed rats at the conclusion of 7 week feeding period. We found no correlation between immune function and development of preneoplastic colon lesions in this study.

30. Biochem Soc Trans 1997 Nov;25(4):S619

Quercetin exhibits hepatoprotective activity in rats.

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31. Biochem Soc Trans 1997 Nov;25(4):S581

Quercetin protects against hydrogen peroxide--induced cataract.

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32. Biomed Pharmacother 1997;51(8):305-10

Absorption, metabolism and health effects of dietary flavonoids in man.

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Flavonoids are polyphenolic compounds that occur ubiquitously in foods of plant origin. Over 4,000 different flavonoids have been described, and they are categorized into flavonols, flavones, catechins, flavanones, anthocyanidins and isoflavonoids. Flavonoids have a variety of biological effects in numerous mammalian cell systems, in vitro as well in vivo. Recently, much attention has been paid to their antioxidant properties and to their inhibitory role in various stages of tumour development in animal studies. Quercetin, the major representative of the flavonol subclass, is a strong antioxidant, and prevents oxidation of low density lipoproteins in vitro. Oxidized low density lipoproteins are atherogenic, and are considered to be a crucial intermediate in the formation of atherosclerotic plaques. This agrees with observations in epidemiological studies that the intake of flavonols and flavones was inversely associated with subsequent coronary heart disease. However, no effects of flavonols on cancer were found in these studies. The extent of absorption of flavonoids is an important unsolved problem in judging their many alleged health effects. Flavonoids present in foods were considered non-absorbable because they are bound to sugars as beta-glycosides. Only free flavonoids without a

sugar molecule, the so-called aglycones, were thought to be able to pass through the gut wall. Hydrolysis only occurs in the colon by microorganisms, which at the same time degrade flavonoids. We performed a study to quantify absorption of various dietary forms of quercetin. To our surprise, the quercetin glycosides from onions were absorbed far better than the pure aglycone. Subsequent pharmacokinetic studies with dietary quercetin glycosides showed marked differences in absorption rate and bioavailability. Absorbed quercetin was eliminated only slowly from the blood. The metabolism of flavonoids has been studied frequently in various animals, but very few data in humans are available. Two major sites of flavonoid metabolism are the liver and the colonic flora. There is evidence for O-methylation, sulphation and glucuronidation of hydroxyl groups in the liver. Bacterial ring fission of flavonoids occurs in the colon. The subsequent degradation products, phenolic acids, can be absorbed and are found in urine of animals. Quantitative data on metabolism are scarce.

33. FEBS Lett 1997 Nov 24;418(1-2):152-6

Relative bioavailability of the antioxidant flavonoid quercetin from various foods in man.

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Quercetin is a strong antioxidant and a major dietary flavonoid. Epidemiological studies suggest that consumption of quercetin protects against cardiovascular disease, but its absorption in man is controversial. We fed nine subjects a single large dose of onions, which contain glucose conjugates of quercetin, apples, which contain both glucose and non-glucose quercetin glycosides, or pure quercetin-3-rutinoside, the major quercetin glycoside in tea. Plasma levels were then measured over 36 h. Bioavailability of quercetin from apples and of pure quercetin rutinoside was both 30% relative to onions. Peak levels were achieved less than 0.7 h after ingestion of onions, 2.5 h after apples and 9 h after the rutinoside. Half-lives of elimination were 28 h for onions and 23 h for apples. We conclude that conjugation with glucose enhances absorption from the small gut. Because of the long half-lives of elimination, repeated consumption of quercetin-containing foods will cause accumulation of quercetin in blood.

34. FEBS Lett 1997 Jun 2;409(1):12-6

Bioavailability of rutin and quercetin in rats.

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Quercetin is a powerful antioxidant which is widely distributed in edible plants, mainly as glycosides such as rutin. It has been reported to be absorbed in mammals, but its metabolism needs further investigation to evaluate its possible physiological effects. We compared the evolution of the absorption of quercetin and rutin in rats fed with supplemented diets. Rutin was absorbed more slowly than quercetin because it must be hydrolysed by the cecal microflora, whereas quercetin was absorbed from the small intestine. Conjugated derivatives of quercetin, and its methylated forms isorhamnetin and tamarixetin, were recovered in plasma from rats receiving the two kinds of experimental diets after the first meal, but after 10 days, no traces of tamarixetin were detected anymore. The rate of elimination of quercetin metabolites seems very low, and high plasma concentrations are easily maintained with a regular supply of quercetin or rutin in the diet.

35. Cancer Lett 1997 Mar 19;114(1-2):141-4

Consumption of quercetin and kaempferol in free-living subjects eating a variety of diets.

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Quercetin and flavonoids are anticarcinogenic in rats, but little is known about human intakes. The intake of five major flavonols and flavones was calculated using 1-day dietary records of 17 volunteers from 14 countries, and using both 3-day records and a food frequency questionnaire of eight Dutch adults. Total consumption (+/- SD) was 27.6 +/- 19.5 mg/day in the international subjects, 34.1 +/- 31.2 mg/day in the Dutch adults according to 3-day records, and 41.9 +/- 23.7 mg/day according to questionnaires. Quercetin contributed 68-73%, and kaempferol 22-29%, the major sources being tea and onions. A brief food frequency questionnaire may be a suitable method for ranking individuals by flavonol intake.

36. Cancer Lett 1997 Mar 19;114(1-2):107-8

Flavonoids as bioactive components in apple products.

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This research focuses on determining the concentration and antioxidant activity of flavonoids in apples and apple products as a function of storage and processing. The results will be used to optimise apple products with respect to both flavonoid content and antioxidant activity.

37. Oncology 1997 Mar-Apr;54(2):118-21

Effects of quercetin and/or restraint stress on formation of aberrant crypt foci induced by azoxymethane in rat colons.

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The present study examines the effect of dietary quercetin and/or restraint stress on the formation of aberrant crypt foci (ACF) induced by azoxymethane (AOM) in the colon. Female F344 rats were divided into four groups. All animals were given intraperitoneal injections of AOM. The animals were fed a basal diet (group A, C), or a 2% quercetin-supplemented diet (group B, D). The animals were put individually to narrow wire cages for 1 h every day throughout the study to expose them to mild restraint stress (group C, D). At week 5, all the rats were killed and analyzed for ACF in the colon. The number of ACF increased significantly in the animals subjected to stress ($p < 0.05$). On the other hand, dietary quercetin significantly reduced the number of ACF in both the nonstress ($p < 0.001$) and stress groups ($p < 0.05$). These findings suggest that quercetin might have a potential as a chemopreventive drug for colon cancer despite stress.

38. Nutr Cancer 1997;28(1):14-9

Dietary fiber and risk of breast cancer: a case-control study in Uruguay.

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To examine whether dietary fiber modifies breast cancer risk, a case-control study involving 351 newly diagnosed patients with breast cancer and 356 hospitalized controls was conducted in Uruguay. Dietary patterns were assessed in detail by use of a food frequency questionnaire on 64 items, which allowed the calculation of total energy intake. Nutrient residuals were calculated through regression analysis. After adjustment for potential confounders (which included age, residence, family history of breast cancer, prior history of benign breast disease, parity, total energy, red meat, lutein/zeaxanthin and quercetin intake, and menopausal status), dietary fiber and total nonstarch polysaccharides were associated with a strong reduction in risk of breast cancer (odds ratio for uppermost quartile of total dietary fiber = 0.51, 95% confidence limit = 0.31-0.82). Also the dose-response pattern was highly significant ($p < 0.001$). The inverse association was observed in pre- and post-menopausal women and was similar for soluble and insoluble fiber. Furthermore, dietary fiber displayed a strong joint effect with fat, quercetin, and lutein/zeaxanthin.

39. Carcinogenesis 1996 Oct;17(10):2193-200

Dietary phenolics as anti-mutagens and inhibitors of tobacco-DNA adduction in the urothelium of smokers.

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Human urine is known to contain substances that strongly inhibit bacterial mutagenicity of aromatic and heterocyclic amines in vitro. The biological relevance of these anti-mutagens was examined by comparing levels of tobacco-DNA adducts in exfoliated urothelial cells from smokers with the anti-mutagenic activity in corresponding 24-h urine samples. An inverse relationship was found between the inhibition of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP)-mutagenicity by urine extracts in vitro and two DNA adduct measurements: the level of the putatively identified N-(deoxyguanosine-8-yl)-4-aminobiphenyl adduct and the total level of all tobacco-smoke-carcinogen adducts including those probably derived from PhIP. Urinary anti-mutagenicity in vitro appears thus to be a good indicator of the anti-genotoxicity exerted by substances excreted in urine, that protect the bladder mucosal cells (and possibly other cells) against DNA damage. These

substances appear to be dietary phenolics and/or their metabolites because (i) the anti-mutagenic activity of urine extracts (n = 18) was linearly to their content in phenolics; (ii) the concentration ranges of these substances in urine extracts were similar to those of various plant phenols (quercetin, isorhamnetin and naringenin) for which an inhibitory effect on the liver S9-mediated mutagenicity of PhIP was obtained; (iii) treatment of urines with beta-glucuronidase and arylsulfatase enhanced both anti-mutagenicity and the levels of phenolics in urinary extracts; (iv) urinary extracts inhibited noncompetitively the liver S9-mediated mutagenicity of PhIP as did quercetin, used as a model phenolics. Several structural features of the flavonoids were identified as necessary for the inhibition of PhIP and 2-amino-3,8-dimethylimidazo[4,5-f]quinoxiline mutagenicity. Fractionation by reverse-phase HPLC and subsequent analysis of two urinary extracts, showed the presence of several anti-mutagenic substances and phenolics; more lipophilic phenolics displayed the highest specific inhibitory activity. This suggests that enzymatic conversion of dietary flavonoids into their more lipophilic and anti-mutagenic O-methylcatechol derivatives, as noted for quercetin, may occur in vivo in man. Onion, lettuce, apples and red wine are important sources of dietary flavonoids which are probably responsible for the anti-mutagenicity associated with foods and beverages. After HPLC fractionation of urinary extracts, the distribution profile of anti-mutagenic activity corresponded roughly to that of onion and wine extract combined. Our study strongly suggests that smokers ingesting dietary phenolics, probably flavonoids, are partially protected against the harmful effects by tobacco carcinogens within their bladder mucosal cells. This protective effect of dietary phenolics against the cancer of the bladder (and possibly other sites) should be verified and explored as a part of a chemoprevention strategy.

40. J Pharmacol Exp Ther 1996 Aug;278(2):771-9

Effect of quercitrin on acute and chronic experimental colitis in the rat.

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Quercitrin was tested for acute and chronic anti-inflammatory activity in trinitrobenzenesulfonic acid-induced rat colitis. The inflammatory status was evaluated by myeloperoxidase, alkaline phosphatase and total

glutathione levels, leukotriene B4 synthesis, in vivo colonic fluid absorption, macroscopical damage and occurrence of diarrhea and adhesions. Treatment with 1 or 5 mg/kg of quercitrin by the oral route reduced myeloperoxidase and alkaline phosphatase levels, preserved normal fluid absorption, counteracted glutathione depletion and ameliorated colonic damage at 2 days. Increasing or lowering the dose of the flavonoid resulted in marked loss of effect. The acute anti-inflammatory effect of quercitrin is unto impairment of neutrophil function or lipoxygenase inhibition, and it may be caused by mucosal protection or enhancement of mucosal repair secondary to increased defense against oxidative insult and/or preservation of normal colonic absorptive function. When tested in chronic colitis (2 and 4 weeks), quercitrin treatment (1 or 5 mg/kg.day) decreased colonic damage score and the incidence of diarrhea, and normalized the colonic fluid transport. All other parameters were unaffected. The chronic effect of the flavonoid is apparently to its action on colonic absorption, although it can be partly secondary to its acute beneficial effect.

41. Mutat Res 1996 Jul 10;369(1-2):97-106

Inhibition by quercetin and luteolin of chromosomal alterations induced by salted, deep-fried fish and mutton in rats.

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Previous studies from our laboratory have shown the clastogenic effects of long-term feeding on deep-fried fish and mutton in rat bone marrow cells. We report the chemopreventive action of two flavanoids, quercetin (Qn) and luteolin (Ln) against the induced mutagenicity by fish and mutton extracts. Groups of rats were treated with flavanoids through pre-, simultaneous- and post-treatment regimens and killed at the end of treatment. The bone marrow was removed and analysed for the presence of micronuclei (MN) and chromosome aberrations (CA). Pre-treatment showed most effectively a good inhibition of mutagenicity at every dose tested. Luteolin was a better protective agent than quercetin. It protected the cells against genetic damage to 93% in the micronucleus assay and to 95% in the chromosome aberrations induced by fish extract ($p < 0.001$ in both the groups). Mutton extract-induced micronuclei and chromosome aberrations were protected 85% and 90%, respectively, by luteolin and 79% and 76%, respectively, by quercetin. Our results tend to suggest that quercetin and luteolin are potential chemopreventive compounds.

42. Planta Med 1996 Jun;62(3):222-6

Inhibition of bacterial mutagenesis by Citrus flavonoids.

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The antimutagenicity of the Citrus flavonoids naringin, hesperidin, nobiletin, and tangeretin against the mutagens benzo[a]pyrene, 2-aminofluorene, quercetin, and nitroquinoline N-oxide was investigated in the Salmonella/microsome assay. Naringin and hesperidin showed a weak antimutagenic activity against benzo[a]pyrene. Tangeretin was antimutagenic against all indirectly-acting mutagens tested, but in general a large molar excess was necessary. Liquid preincubation increased the antimutagenicity of tangeretin against 2-aminofluorene. Nobiletin acted as an antimutagen against benzo[a]pyrene, but it enhanced the mutagenicity of 2-aminofluorene. However, in a liquid preincubation assay nobiletin also exhibited antimutagenicity against 2-aminofluorene. Both tangeretin and nobiletin inhibited the mutagenicity of quercetin. Quercetin itself acted as an antimutagen against 2-aminofluorene in a Salmonella strain (TA1538) where its mutagenicity was not expressed. Quercetin should not merely be regarded as a genotoxic risk factor in the human diet, since its mutagenicity may be inhibited by accompanying compounds including other flavonoids, and since quercetin itself also exhibits an antimutagenic action. Because of the antimutagenic properties the Citrus flavonoids tested, especially tangeretin and nobiletin, might play a role in the chemoprevention of cancer.

43. Carcinogenesis 1996 Jun;17(6):1305-11

Effects of the phytochemicals, curcumin and quercetin, upon azoxymethane-induced colon cancer and 7,12-dimethylbenz[a]anthracene-induced mammary cancer in rats.

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Curcumin and quercetin were evaluated in rats for their ability to modulate the carcinogenic activity of azoxymethane (AOM) in the colon and 7,12-dimethylbenz[a]anthracene (DMBA) in the mammary gland. In the AOM-induced colon cancer model, male Fischer 344 rats at 8 weeks of age

started to receive either curcumin (8 and 16 g/kg) or quercetin (16.8 and 33.6 g/kg) in the diet and 1 week later, were administered AOM (30 mg/kg body wt.) by subcutaneous injection. The animals continued to receive the two agents in the diet until sacrificed 45 weeks later. Curcumin mediated a dose-dependent inhibition of the incidence and multiplicity of adenomas from 47% and 0.58 +/- 0.12 adenomas/rat in the AOM-treated control group to 19% and 0.22 +/- 0.08 and 0.06% and 0.08 +/- 0.06 adenomas/rat for the low and high dose groups, respectively. A low yield of adenocarcinomas (0.06 +/- 0.04 adenocarcinomas/rat) was induced by AOM which was not significantly altered by curcumin. Treatment with quercetin caused a dose-dependent increase in the yield of AOM-induced tumors in the colon from 0.06 +/- 0.04 adenocarcinoma/rat to 0.64 +/- 0.12 and 1.14 +/- 0.17 for the low and high dose groups, respectively. In the DMBA-induced mammary cancer model, curcumin or quercetin was administered at either 10 or 20 g/kg diet, beginning 7 days prior to DMBA and continually throughout the remainder of the experiment. Neither curcumin nor quercetin significantly altered the incidence of animals with tumors or the tumor multiplicity, while the high concentration of both agents significantly increased tumor latency. These results demonstrate different responses to these agents in the two models. While curcumin was highly effective as a chemopreventive agent in the colon model, it was only weakly effective in the mammary model. In contrast, quercetin which was also only weakly effective in the mammary model, caused a dose-dependent enhancement of tumors induced by AOM in the colon model.

44. Int J Cancer 1996 May 3;66(3):404-8

Effect of quercetin on the genotoxic potential of cisplatin.

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The natural product flavonoid quercetin has been shown to sensitise cells to the cytotoxic potential of cisplatin. Both cisplatin and quercetin are genotoxicants. As quercetin is currently in clinical trial as a cytotoxicant-sensitising agent, we wanted to elucidate whether it affects the genotoxicity associated with cisplatin. The genotoxic potential of both agents alone and in combination was studied in Salmonella typhimurium strains TA 98, TA 100 and TA 102 and by assessment of unscheduled DNA synthesis (UDS) in rat hepatocytes. Furthermore, effects of quercetin on levels of cisplatin-DNA adducts were studied in hepatocytes by ELISA. Cisplatin was mutagenic in all 3

bacterial strains and quercetin in strain TA 98. The number of revertant Salmonella colonies observed with the combination did not differ significantly from that caused by the drugs on their own. In the UDS assay, cisplatin was genotoxic but quercetin was not. In combination, quercetin decreased the nuclear grain count caused by cisplatin, but quercetin did not alter the level of cisplatin-DNA adduct formation in hepatocytes. Our results suggest that the mutagenic potential of the combination cisplatin-quercetin, as judged by the bacterial short-term test, does not exceed that associated with the individual components. However, in hepatocytes, quercetin appears to inhibit repair of cisplatin-induced DNA damage. Therefore, in patients who are to be treated with a combination of cisplatin and quercetin, the risk of genotoxicity in normal tissues will have to be taken into consideration.

45. Carcinogenesis 1996 Apr;17(4):877-9

Inhibitory effect of dietary flavonol quercetin on 7,12-dimethylbenz[a]anthracene-induced hamster buccal pouch carcinogenesis.

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The inhibitory effect of dietary supplementation with flavonol quercetin on 7,12-dimethylbenz[a]anthracene (DMBA)-induced hamster buccal pouch carcinogenesis was investigated. Dietary quercetin inhibited the incidence of both papillomas and tumors induced by DMBA. The fluorescence spectra of papillomas and tumors showed different prominent maxima and a characteristic peak around 620-630 nm, which could be attributed to the accumulation of porphyrin compounds. Further, the fluorescence intensities at 630 nm (FI630nm) were elevated, whereas the ratio FI530nm/FI630nm was decreased in DMBA-induced lesions. Quercetin treatment significantly decreased FI630nm and increased the ratio FI520nm/FI630nm when compared with DMBA-induced lesions. It is therefore evident that quercetin has an inhibitory effect on DMBA-induced carcinogenesis and further studies will throw more light on its use as a chemopreventive agent against oral cancer.

46. Chem Phys Lipids 1996 Mar 29;79(2):157-63

Antioxidant activity of natural flavonoids is governed by number and location of their aromatic hydroxyl groups.

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We studied antioxidant activity of five natural flavonoids in canola oil, an emulsion, and rat red blood cell membrane. In the canola oil heated at 105 degrees C, myricetin most effectively attenuated lipid oxidation, followed by quercetin, morin and kaempferol in a decreasing order. When the emulsion containing canola oil was incubated at 54 degrees C, quercetin was most protective against lipid oxidation, followed by kaempferol, myricetin and morin in a decreasing rank. Apigenin demonstrated no antioxidant activity in either the canola oil or the emulsion. Of the flavonoids tested, quercetin was the best in quenching free radical chain reaction in rat red blood cell membrane. The antioxidative characteristics of these flavonoids was determined by multiple factors including the system used, the hydrophobicity, and the total number and the location of aromatic hydroxyl groups. We conclude that myricetin is an effective antioxidant in canola oil, while quercetin is an effective protector against lipid oxidation in the cell membrane.

47. Arch Intern Med 1996 Mar 25;156(6):637-42

Dietary flavonoids, antioxidant vitamins, and incidence of stroke: the Zutphen study.

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BACKGROUND: Epidemiological studies suggested that consumption of fruit and vegetables may protect against stroke. The hypothesis that dietary antioxidant vitamins and flavonoids account for this observation is investigated in a prospective study. METHODS: A cohort of 552 men aged 50 to 69 years was examined in 1970 and followed up for 15 years. Mean nutrient and food intake was calculated from cross-check dietary histories taken in 1960, 1965, and 1970. The association between antioxidants, selected foods, and stroke incidence was assessed by Cox proportional hazards regression analysis. Adjustment was made for confounding by age, systolic blood pressure, serum cholesterol, cigarette smoking, energy intake, and consumption of fish and alcohol. RESULTS: Forty-two cases of first fatal or nonfatal stroke were documented. Dietary flavonoids (mainly quercetin) were inversely

associated with stroke incidence after adjustment for potential confounders, including antioxidant vitamins. The relative risk (RR) of the highest vs the lowest quartile of flavonoid intake (> or = 28.6 mg/d vs <18.3 mg/d) was 0.27 (95% confidence interval [CI], 0.11 to 0.70). A lower stroke risk was also observed for the highest quartile of beta-carotene intake (RR, 0.54; 95% CI, 0.22 to 1.33). The intake of vitamin C and vitamin E was not associated with stroke risk. Black tea contributed about 70% to flavonoid intake. The RR for a daily consumption of 4.7 cups or more of tea vs less than 2.6 cups of tea was 0.31 (95% CI, 0.12 to 0.84). CONCLUSION: The habitual intake of flavonoids and their major source (tea) may protect against stroke.

48. Exp Hematol 1996 Mar;24(4):494-6

Concentration and effect of quercetin.

Larocca LM, Piantelli M, Teofili L, Leone G, Ranelletti FO

49. Eur J Clin Nutr 1996 Feb;50(2):63-71

Potential health effects of the dietary flavonol quercetin.

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50. Nutr Cancer 1996;26(2):167-81

Inhibition of human breast cancer cell proliferation and delay of mammary tumorigenesis by flavonoids and citrus juices.

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Two citrus flavonoids, hesperetin and naringenin, found in oranges and grapefruit, respectively, and four noncitrus flavonoids, baicalein, galangin, genistein, and quercetin, were tested singly and in one-to-one combinations for their effects on proliferation and growth of a human breast carcinoma cell line, MDA-MB-435. The concentration at which cell proliferation was inhibited by 50% (IC50), based on incorporation of [3H]thymidine, varied from 5.9 to 140 micrograms/ml for the single

flavonoids, with the most potent being baicalein. IC50 values for the one-to-one combinations ranged from 4.7 micrograms/ml (quercetin + hesperetin, quercetin + naringenin) to 22.5 micrograms/ml (naringenin + hesperetin). All the flavonoids showed low cytotoxicity (> 500 micrograms/ml for 50% cell death). Naringenin is present in grapefruit mainly as its glycosylated form, naringin. These compounds, as well as grapefruit and orange juice concentrates, were tested for their ability to inhibit development of mammary tumors induced by 7,12-dimethylbenz[a]anthracene (DMBA) in female Sprague-Dawley rats. Two experiments were conducted in which groups of 21 rats were fed a semipurified diet containing 5% corn oil and were given a 5-mg dose of DMBA intragastrically at approximately 50 days of age while in diestrus. One week later, individual groups were given double-strength grapefruit juice or orange juice or fed naringin or naringenin at levels comparable to that provided by the grapefruit juice; in the second experiment, the rats were fed a semipurified diet containing 20% corn oil at that time. As expected, rats fed the high-fat diet developed more tumors than rats fed the low-fat diet, but in both experiments tumor development was delayed in the groups given orange juice or fed the naringin-supplemented diet compared with the other three groups. Although tumor incidence and tumor burden (grams of tumor/rat) were somewhat variable in the different groups, rats given orange juice had a smaller tumor burden than controls, although they grew better than any of the other groups. These experiments provide evidence of anticancer properties of orange juice and indicate that citrus flavonoids are effective inhibitors of human breast cancer cell proliferation in vitro, especially when paired with quercetin, which is widely distributed in other foods.

51. Food Chem Toxicol 1995 Dec;33(12):1061-80

Review of the biology of Quercetin and bioflavonoids.

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The French paradox is a dietary anomaly which has focused attention on the Mediterranean diet. Epidemiological studies revealed that this diet, replete in flavonoid-rich foods (Allium and Brassica vegetables, and red wine), correlate with the increased longevity and decreased incidence of cardiovascular disease seen in these populations. The most frequently studied flavonoid, quercetin, has been shown to have biological

properties consistent with its sparing effect on the cardiovascular system. Quercetin and other flavonoids have been shown to modify eicosanoid biosynthesis (antiprostanoid and anti-inflammatory responses), protect low-density lipoprotein from oxidation (prevent atherosclerotic plaque formation), prevent platelet aggregation (antithrombic effects), and promote relaxation of cardiovascular smooth muscle (antihypertensive, antiarrhythmic effects). In addition, flavonoids have been shown to have antiviral and carcinostatic properties. However, flavonoids are poorly absorbed from the gut and are subject to degradation by intestinal micro-organisms. The amount of quercetin that remains biologically available may not be of sufficient concentration, theoretically, to explain the beneficial effects seen with the Mediterranean diet. The role of flavonoids may transcend their presence in food. The activity of flavonoids as inhibitors of reverse transcriptase suggests a place for these compounds in the control of retrovirus infections, such as acquired immunodeficiency syndrome (AIDS). In addition to specific effects, the broad-modulating effects of flavonoids as antioxidants, inhibitors of ubiquitous enzymes (ornithine carboxylase, protein kinase, calmodulin), and promoters of vasodilatation and platelet disaggregation can serve as starting material for drug development programmes.

52. Cancer Lett 1995 Jul 20;94(1):113-21

Modulating effects of ellagic acid, vanillin and quercetin in a rat medium term multi-organ carcinogenesis model.

Akagi K, Hirose M, Hoshiya T, Mizoguchi Y, Ito N, Shirai T
First Department of Pathology, Nagoya City University, Medical School, Japan.

Effects of dietary supplementation with the antioxidants ellagic acid, quercetin and vanillin were examined using a medium term multi-organ carcinogenesis model in rats. Groups of 10-15 male F344 rats were given i.p. injections of diethylnitrosamine (DEN, 100 mg/kg body wt.) and N-methylnitrosourea (MNU, 20 mg/kg body wt), s.c. injections of 1,2-dimethylhydrazine (DMH, 40 mg/kg body wt.), together with 0.05% N-butyl-N-(4- hydroxybutyl)nitrosamine (BBN) and 0.1% 2,2'-dihydroxy-di-n-propylnitrosamine (DHPN), both in the drinking water, for a total multiple initiation period of 4 weeks (DMBDD) treatment). Ellagic acid, quercetin or vanillin, each at a dose of 1% each in the diet were administered from 1 day before and throughout the carcinogen exposure period, or after completion of the initiation

regimen. All surviving animals were sacrificed at the end of week 36, and major organs were examined histopathologically. In the small intestine, significant reductions in the incidence and number of tumors (adenomas and carcinomas) were observed in the groups administered ellagic acid during (8%, 0.08 +/- 0.29) or after (8%, 0.08 +/- 0.29) DMBDD treatment, and those receiving quercetin after DMBDD treatment (0%) compared to the control value (57%, 1.07 +/- 1.21). Although the incidences were not statistically significant, slightly decreased numbers of small intestinal tumors were found in the groups receiving vanillin during (0.33 +/- 0.72), or after (0.40 +/- 0.83) DMBDD treatment. The incidence of large intestinal carcinomas in the group treated with vanillin during DMBDD treatment was significantly higher (73%) than the control value (21%). These results indicated that while ellagic acid and quercetin exerted potent chemopreventive action in both the initiation and promotion stages in the present experimental system, their beneficial effects were restricted to the small intestine. Since small intestinal carcinomas are very infrequent in humans, the advantages of these phenolic compounds for human application as chemopreventors should not be overestimated.

53. Biochem Mol Biol Int 1995 Jul;36(3):545-50

Quercetin, a bioflavonoid, inhibits the DNA synthesis of human leukemia cells.

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Quercetin, a flavonoid, is found in many plants, including edible fruits and vegetables. It has been proposed that flavonoids may have potential as anticancer agents. To test an aspect of this hypothesis, we examined the effects of the flavonoid, quercetin, on the DNA synthesis of the human leukemia cell, HL-60. Quercetin induced a dose-dependent inhibition of DNA synthesis in the test range of 1 microM to 1 mM. The inhibitory effect on DNA synthesis was evident as early as 24 h after the addition of quercetin. At the concentrations of 10 microM, 100 microM and 1 mM, 50, 82 and 85% of DNA synthesis, respectively, was inhibited by quercetin as compared to the control. After 48 and 72 h incubation of the cells with 100 microM and 1 mM quercetin, DNA synthesis was almost completely abolished. These results suggest that the inhibitory effects of quercetin on HL-60 cell DNA synthesis is not due to a non-specific cytotoxic effect, since following removal of

quercetin, the treated cells regrew normally.

54. Blood 1995 Jun 15;85(12):3654-61

Quercetin inhibits the growth of leukemic progenitors and induces the expression of transforming growth factor-beta 1 in these cells.

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We previously showed that quercetin (3,3',4',5,7 pentahydroxyflavone) inhibits in a dose-dependent manner the growth of acute leukemias and is able to enhance the antiproliferative activity of cytosine arabinoside. We show here that quercetin inhibits the clonogenic activity of 20 of 22 acute leukemias (AL; 4 M1-AML, 3 M2-AML, 2 M3-AML, 3 M4-AML, 3 M5-AML, and 7 ALL). In the present report, we show that the induction of transforming growth factor-beta 1 (TGF-beta 1) in leukemic blasts is one of the growth-inhibitory mechanisms of quercetin in these cells. This observation was supported by the following data. (1) Quercetin-sensitive leukemic blasts, when treated with quercetin, secrete large amounts of TGF-beta 1 in the medium and show positivity for TGF-beta 1-immunoreactive material in the cytoplasm. (2) At a concentration of 8 mumol/L, antisense TGF-beta 1 oligonucleotides prevent the growth-inhibitory action of quercetin. (3) Anti-TGF-beta 1 neutralizing monoclonal antibodies can prevent almost completely the growth-inhibitory activity of quercetin. The analysis of quercetin-resistant cases confirmed as well the central role of TGF-beta 1 in the growth-inhibitory activity of quercetin. In conclusion, quercetin can act as a cytostatic agent for leukemic cells by modulating the production of TGF-beta 1.

55. Mutat Res 1995 Jun;343(2-3):85-94

Genotoxicity of quercetin in the micronucleus assay in mouse bone marrow erythrocytes, human lymphocytes, V79 cell line and identification of kinetochore-containing (CREST staining) micronuclei in human lymphocytes.

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Quercetin, a mutagenic flavonoid widely distributed in edible plants, was studied for the induction of micronuclei (MN). We have carried out the MN assay in bone marrow polychromatic erythrocytes in mice, in cytokinesis-blocked human lymphocytes and in cytokinesis-blocked V79 cells. MN assay in vitro was performed in the presence and in the absence of S9. To further extend the study, an antikinetochore antibody (CREST staining) was used to distinguish MN containing whole chromosomes (kinetochore positive) from those containing acentric fragments (kinetochore negative). When tested in vivo quercetin failed to induce micronuclei, a result which is in agreement with other published reports. When tested in vitro in V79 cells quercetin clearly induces micronuclei in the absence of S9 and also in the presence of S9 for the highest dose used. When tested in vitro in human lymphocytes quercetin shows a significant induction of micronuclei in the absence and in the presence of S9. The presence of S9 compared to its absence is not significant for any of the systems used. Both in the presence and absence of S9, quercetin appears to behave as a clastogenic agent in human lymphocytes inducing a significant majority of kinetochore-negative MN.

56. Ann Pharmacother 1995 Jun;29(6):627-8

Dietary flavonoids in atherosclerosis prevention.

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More in vivo studies are needed to determine conclusively whether flavonoids inhibit the growth of atherosclerotic plaques, thus reducing the risk of atherosclerosis. In vitro data are contradictory, as the doses used may not be representative of the typical dietary intake of flavonoids, and not enough in vivo data exist to make conclusive statements. Exactly which flavonoid(s) may cause therapeutic benefit remains unclear because quercetin does not appear to be systemically absorbed, and myricetin and gossypetin have the potential to increase uptake of LDL by macrophages. More pharmacokinetic studies are needed to determine the bioavailability of each major dietary flavonoid. In vitro studies have shown that flavonoids possess potent antioxidant activity, which may slow oxidative modification of LDLs. Additional studies similar to the Zutphen Elderly Study and controlled clinical studies are needed, with more accurate estimates of daily flavonoid intake to determine conclusively whether a high-flavonoid diet reduces the rate of

atherosclerosis. Until those studies are completed, consuming flavonoids cannot be recommended as a means to reduce the risk of atherosclerosis. Instead, one should focus on limiting the amount of saturated fats in the diet, smoking cessation, and participating in physical exercise to reduce risk of atherosclerosis.

57. Free Radic Res 1995 Apr;22(4):375-83

The relative antioxidant activities of plant-derived polyphenolic flavonoids.

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The relative antioxidant activities, against radicals generated in the aqueous phase, of a range of plant-derived polyphenolic flavonoids, constituents of fruit, vegetables, tea and wine, have been assessed. The results show that compounds such as quercetin and cyanidin, with 3',4' dihydroxy substituents in the B ring and conjugation between the A and B rings, have antioxidant potentials four times that of Trolox, the vitamin E analogue. Removing the ortho-dihydroxy substitution, as in kaempferol, or the potential for electron delocalisation by reducing the 2,3 double bond in the C ring, as in catechin and epicatechin, decreases the antioxidant activity by more than 50%, but these structures are still more effective than alpha-tocopherol or ascorbate. The relative significance of the positions and extents of hydroxylation of the A and B rings to the total antioxidant activity of these plant polyphenolics is demonstrated.

58. Cell Mol Biol Res 1995;41(6):551-60

Suppression of oncogene-induced transformation by quercetin and retinoic acid in rat liver epithelial cells.

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AP1 is a heterodimeric complex containing products of the Jun and Fos oncogene families. The c-fos and c-jun protooncogenes act as transcriptional activator for numerous cellular genes, and the overexpression of these genes may cause malignant transformation. In this study, to show evidence of a possible inhibition of AP1 transcriptional activity in molecular mechanisms of foodborne molecules, known to be negative modulators of carcinogenesis, we established two rat liver epithelial (REL) cell lines overexpressing either c-fos (43C line) or c-jun (RELcJ1 line) oncoproteins. Contrary to the 43C line, which was spontaneously transformed, the c-jun-transfected REL cells were only transformed in vitro after 12-O-tetra-decanoylphorbol

13-acetate (TPA) exposure. All trans-retinoic acid (RA) abolished the transformation of the 43C line and TPA-treated RELcJ1 cells, suggesting that RA could decrease AP1 activity in these cells despite c-fos or c-jun overexpression. Furthermore, we show for the first time that a flavonoid, quercetin, which is a natural component of vegetables, inhibited only the transformation of the 43C line. The spontaneous transformation of the c-fos-transfected REL cells was associated with the appearance of c-fos/AP1 complexes binding TRE, suggesting that c-fos/AP1 complexes are involved in the antitransforming mechanism of quercetin.

59. Eur J Cancer Prev 1994 Dec;3 Suppl 2:13-7

Genotoxic flavonoids and red wine: a possible role in stomach carcinogenesis.

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60. Cancer Lett 1994 Nov 25;87(1):107-13

Published erratum appears in Cancer Lett 1995 Jan 6;88(1):119-20

Chemopreventive potential of dietary bioflavonoids against 20-methylcholanthrene-induced tumorigenesis.

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The effect of dietary supplementation of flavonoidal compounds such as quercetin, rutin, luteolin and (+)-catechin on the incidence of fibrosarcoma induced by 20-methylcholanthrene (20-MC) in male Swiss albino mice was observed. Subcutaneous injection of 20-MC produced 100% tumor incidence and the onset of tumor appeared within 7 weeks, while flavonoid-treated mice (1% quercetin- and luteolin-mixed diets) produced tumors in the 9th week, and the tumor incidences in mice treated with quercetin- and luteolin-mixed diets were 52% and 60%, respectively. Subcutaneous administration of 20-MC along with the flavonoidal compounds (quercetin, luteolin) was found to have significant effect on tumor expression. The compounds rutin and (+)-catechin did not influence tumor expression in both experiments. Elevated levels of lipid peroxides, cytochrome P450 and decreased activity of

glutathione-S-transferase (GST) were observed in the tumor bearing animals. Test-diet-treated animals showed reduction in the lipid peroxides and cytochrome P450, and increased activity of GST ($P < 0.001$). In vitro [3H]thymidine incorporation showed the inhibition of DNA synthesis in fibrosarcoma cells by the flavonoids. The possible mode of action of the flavonoidal compounds may be through their influence on the initiation and promotion phases of the carcinogenic process coupled with enhancement of the detoxification process.

61. Carcinogenesis 1994 Nov;15(11):2669-72

Radioprotective effect of antioxidative flavonoids in gamma-ray irradiated mice.

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The anticlastogenic effect of 12 structurally different flavonoids was investigated in whole body gamma-ray irradiated mice. Each flavonoid was administered to ICR male mice by a single gastric intubation (5 mumol/kg) 6 h before gamma-ray irradiation (1.5 Gy) and the frequency of micronucleated reticulocytes (MNRETs) in peripheral blood was determined. In order to elucidate the mechanism of the anticlastogenic effect of these flavonoids, their antioxidative activities were examined by the thiobarbituric acid method using methyl linoleate and Fenton's reagent ($Fe2+/H2O2$). Of the 12 flavonoids, luteolin had the most marked effect on reducing the frequencies of MNRETs and also inhibiting lipid peroxidation. However, quercetin tetramethylether, which has methoxy groups instead of hydroxyl groups at the 3,7,3',4'-positions, and phloretin with an open C-ring showed the least anticlastogenic and antioxidative activity. A good correlation ($r = 0.717$, $P < 0.01$) was observed between the anticlastogenic activity and the antioxidative activity of the 12 flavonoids. These results suggest that the radioprotective effect of flavonoids in mice may be attributed to the hydroxyl radical scavenging potency in a direct or an endogenous enzyme mediated manner.

62. J Dairy Res 1994 Nov;61(4):545-52

Inhibitory effect of dairy products on the mutagenicities of chemicals and dietary mutagens.

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The antimutagenic effects of uninoculated milk and milks cultured with Bifidobacterium or Lactobacillus strains towards the mutagenicity induced by two direct mutagens, 4-nitroquinoline N-oxide and 2-nitrofluorene, and three dietary indirect mutagens, aflatoxin B1, benzo(a)pyrene and quercetin, were investigated using the in vitro Salmonella typhimurium test. Each cultured milk sample and control milk had a significant antimutagenic effect, to an extent varying with the mutagen used. Uninoculated milk had a greater inhibitory effect than cultured milks towards dietary indirect mutagens.

63. J Pharm Pharmacol 2000 Apr;52(4):451-9

Identification of kaempferol as a monoamine oxidase inhibitor and potential Neuroprotectant in extracts of Ginkgo biloba leaves.

Sloley BD, Urichuk LJ, Morley P, Durkin J, Shan JJ, Pang PK, Coutts RT
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The effects of Ginkgo biloba leaf extract on rat brain or livermonoamine oxidase (MAO)-A and -B activity, biogenic amine concentration in nervous tissue, N-methyl-D-aspartate (NMDA)- and N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4)-induced neurotoxicity and antioxidant activity was investigated to determine the effects of the extract on monoamine catabolism and neuroprotection. Ginkgo biloba leaf extract was shown to produce in-vitro inhibition of rat brain MAO-A and -B. The Ginkgo biloba extract was chromatographed on a reverse-phase HPLC system and two of the components isolated were shown to be MAO inhibitors (MAOIs). These MAOIs were identified by high-resolution mass spectrometry as kaempferol and isorhamnetin. Pure kaempferol and a number of flavonoids were examined as MAOIs in-vitro. Kaempferol, apigenin and chrysin proved to be potent MAOIs, but produced more pronounced inhibition of MAO-A than MAO-B. IC50 (50% inhibition concentration) values for the ability of these three flavones to inhibit MAO-A were 7 x 10(-7), 1 x 10(-6) and 2 x 10(-6) M, respectively. Ginkgo biloba leaf extract and kaempferol were found to have no effect ex-vivo on rat or mouse brain MAO or on concentrations of dopamine, noradrenaline, 5-hydroxytryptamine and 5-hydroxyindoleacetic acid. Kaempferol was shown to protect against NMDA-induced neuronal toxicity in-vitro in rat cortical cultures, but did not prevent DSP-4-induced noradrenergic neurotoxicity in an in-vivo model. Both Ginkgo biloba extract and kaempferol were demonstrated to be antioxidants in a lipid-peroxidation assay. This data

indicates that the MAO-inhibiting activity of Ginkgo biloba extract is primarily due to the presence of kaempferol. Ginkgo biloba extract has properties indicative of potential neuroprotective ability.

64. Pharmacol Res 2000 Jan;41(1):83-88

Cytotoxic activity of kaempferol glycosides against human leukaemic cell lines in vitro.

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Two kaempferol coumaroyl glycosides (i.e. platanoside and tiliroside) isolated from the methanolic extract of Platanus orientalis L. buds, were examined for their in vitro cytotoxic activity against a panel of human leukaemic cell lines. Platanoside (1) exhibited cytotoxic activity against most of the cell lines tested, while tiliroside (2) was active against two of the nine tested cell lines. Compound 1, was examined for its effect on the uptake of [(3)H]thymidine as a marker of DNA synthesis. Kaempferol was used as a control.

65. J Neurosci Res 1999 Aug 1;57(3):399-404

Phytoestrogen kaempferol (3,4',5,7-tetrahydroxyflavone) protects PC12 and T47D cells from beta-amyloid-induced toxicity.

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In clinical studies, it has been shown that estrogen replacement therapy in menopause is strongly cor with a reduced risk of the development of Alzheimer's disease (AD). In in vitro experiments, it was demonstrated that estradiol protects cells against the toxic effects of beta-amyloid, the major component of plaques in brains of AD patients. Therefore, estrogens have become interesting candidates for a possible treatment of neurodegeneration. In plants, a class of compounds has been identified that bind to human estrogen receptor, so-called phytoestrogens, which are part of our daily diet. Here, we compared the effects of alpha- and beta-estradiol with plant-derived kaempferol on beta-amyloid peptide-induced toxicity in PC12 neuroblastoma and T47D human breast cancer cells. The present results demonstrate a protective effect of kaempferol comparable to that observed with estradiol. The effects of the weak estrogen receptor agonists alpha-estradiol and kaempferol were found to be similar to the effects of

the strong estrogen receptor agonist beta-estradiol, suggesting a mode of action independent from the nuclear estrogen receptor.

66. Arch Pharm Res 1994 Apr;17(2):71-5

Antimutagenic effect of plant flavonoids in the Salmonella assay system.

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The antimutagenic effects of 27 kinds of plant flavonoids on the mutagenicity of aflatoxin B1(AFB1) and N-methyl-N'-nitro-N-nitrosoguanidine(MNNG) in Salmonella typhimurium TA100 were investigated. In the mixed applications of AFB1 (1 microgram/plate) with the flavonoids (300 micrograms/plate) in the presence of a mammalian metabolic activation system (S9 mix), chrysin, apigenin, luteolin and its glucoside, kaempferol, fisetin, morin, naringenin, hesperetin, persicogenin, (+)-catechin and (-)-epicatechin showed the antimutagenic effect against AFB1 with more than 70% inhibition rate. A little or no antimutagenicities except flavone against MNNG (0.5 microgram/plate) were observed. For the antimutagenicity of the flavonoids on AFB1, the flavonoid structure that contains the free 5-, 7-hydroxyl group seemed to be essential. However, saturation of the 2,3-double bond or elimination of the 4-keto group did not affect the activity.

67. Exp Toxicol Pathol 1999 Jan;51(1):9-14

Protective effects of some flavonoids on the renal cellular membrane.

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By assaying lactate dehydrogenase and malondialdehyde leakage from LLC-PK1 cells in culture, a study was conducted to clarify whether flavonoid compounds ameliorate renal cellular injury. The cells were cultured with various concentrations of samples under routine conditions. The results demonstrated that baicalin, cirsimaritin, 6-hydroxyluteolin, luteolin, plantaginin, rhoifolin, sorbarin, afzelin, hyperin, isoquercitrin, isorhamnetin, kaempferitrin,
kaempferol-7-glucoside, oxyayanin A, quercetin, quercitrin, rhamnetin and rutin exerted marked protective effects on the cells, whereas acacetin, apigenin, apiin, cirsilineol, genkwanin, pectolinarin and tetramethylquercitin had virtually no effect. In the light of

these findings, we propose that the general capability of these compounds is largely decided by the number and position of phenolic hydroxyl groups linked to the structural backbone.

68. J Epidemiol 1998 Aug;8(3):168-75

Japanese intake of flavonoids and isoflavonoids from foods.

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The intake of flavonoids and isoflavonoids was estimated based upon a preliminary database of 40 food items, covering at least 80% of total food consumption. Fifty volunteer women in "I-City" recorded the weight of all dietary intake for 3 days in September 1996, and received a health check-up, as well as laboratory examination. The data was analyzed in relation to the various food factors. Average daily intake per capita of flavonoids was as follows: 4.9 mg kaempferol, 8.3 mg quercetin, 1.5 mg rutin, 0.6 mg myricetin, 0.3 mg luteolin, 0.01 mg myricitrin, 0.4 mg fisetin, and 0.3 mg eriodictyol. Total intake from vegetables and fruits was less than 10 mg 16.2 mg (range: 3.18-35.61 mg) and 23.27 mg (4.62-52.12 mg) of isoflavones, such as daidzein and genistein, respectively, were taken per day, and total isoflavone intake was 39.46 mg (7.80-87.73 mg). Chief component analysis on ingested vitamins, flavonoids and isoflavonoids was carried out. Factor 1 was mainly composed of flavonoids and antioxidant vitamins. Factor 1 was positively associated with age and the level of HDL cholesterol and negatively to the level of triglycerides. Factor 2, which was mainly composed of isoflavonoids, was positively associated with creatinine and uric acid levels. So far, these factors did not show a significant association with bone density and other health indices, such as BMI and blood pressure.

69. Nutr Cancer 1999;34(2):160-6

Protection by the flavonoids myricetin, quercetin, and rutin against hydrogen peroxide-induced DNA damage in Caco-2 and Hep G2 cells.

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Flavonoids are reported to exhibit a wide variety of biological effects, including antioxidant and free radical-scavenging activities. Reactive oxygen species have been implicated in a range of human pathological diseases such as atherosclerosis and certain cancers. The aims of this present study were 1) to investigate the effect of the flavonoids myricetin, quercetin, and rutin on cell viability, endogenous antioxidant enzyme activities, and DNA integrity in Caco-2 and Hep G2 cells and 2) to determine whether these flavonoids could protect against H2O2-induced DNA damage. Both cell lines were supplemented with various concentrations (0-200 microM) of myricetin, quercetin, and rutin for 24 hours or H2O2 (50 microM) for 30 minutes, and cell viability was assessed. Over the concentration range tested, neither the flavonoids nor H2O2 significantly affected cell viability. The effect of the flavonoids on the activities of the antioxidant enzymes catalase (EC 1.11.1.6) and superoxide dismutase (EC 1.15.1.1) and on DNA integrity was assessed. The flavonoids did not significantly affect catalase or superoxide dismutase activity and did not induce DNA damage in either cell line. Exposure to 50 microM H2O2 for 30 minutes at 37 degrees C resulted in significant DNA damage, and preincubation with the flavonoids before H2O2 exposure significantly ($p < 0.05$) protected Caco-2 and Hep G2 cells against H2O2-induced DNA damage.

70. Nutr Cancer 1999;34(1):88-99

Quercetin-induced apoptosis in colorectal tumor cells: possible role of EGF receptor signaling.

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Flavonoids are among the best candidates for mediating the protective effect of diets rich in fruits and vegetables with respect to colorectal cancer. To gain additional information about their growth effects on colorectal tumors and their cellular mechanisms of action, a series of flavonoids was added to cultures of colonic tumor cells. Most compounds induced growth inhibition and cell loss at concentrations of 1-100 microM, relative effectivity being quercetin > apigenin > fisetin > robinetin and kaempferol. Myricetin was only slightly effective. Quercetin was the strongest inducer of apoptosis in a process that was reversible until 10 hours by flavonoid removal and until 24 hours by fetal calf serum. Cells were preferentially retained in the S phase. On

the cellular level, quercetin sensitivity was cor with epidermal growth factor (EGF) receptor levels, rapid growth, and poor differentiation, indicating the possibility of targeting those cells most harmful for the organism. The flavonoid transiently inhibited EGF receptor phosphorylation but had only little effect on other signaling molecules. Even after recovery of receptor phosphorylation, cells remained resistant to EGF stimulation. In summary, the data indicate that inhibition of EGF receptor kinase is an integral part of quercetin-induced growth inhibition, but additional mechanisms also contribute to the overall effect.

71. J Cell Biochem 1999 Sep 15;74(4):606-15

Suppression of TNFalpha-mediated NFkappaB activity by myricetin and other flavonoids through downregulating the activity of IKK in ECV304 cells.

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Flavonoids are a group of naturally-occurring phenolic compounds in the plant kingdom, and many flavonoids are found with vascular protective properties. Nevertheless how the protective response is exerted by flavonoids is not well characterized. In view of the nuclear factor-kappaB (NFkappaB) may play a central role in the initiation of atherosclerosis, prevention of the activation of NFkappaB represents an important role in protecting vascular injury. In this study, the effects of flavonoids on NFkappaB/inhibitor-kappaB (IkappaB) system in ECV304 cells activated with tumor necrosis factor-alpha (TNFalpha) were examined. We investigated the inhibitory action of six flavonoids on IkappaB kinase (IKK) activity, an enzyme recently found to phosphorylate critical serine residues of IkappaB for degradation. Of six flavonoids tested, myricetin was found to strongly inhibit IKK kinase activity, and prevent the degradation of IkappaBalpha and IkappaBbeta in activated endothelial cells. Furthermore, myricetin was also found to inhibit NFkappaB activity cor with suppression of monocyte adhesion to ECV304 cells. Therefore we conclude that flavonoids may be of therapeutic value for vascular disease through down regulation of NFkappaB/IkappaB system.

72. Free Radic Biol Med 1999 Jun;26(11-12):1457-66

Repair of iron-induced DNA oxidation by the flavonoid myricetin in primary rat hepatocyte cultures.

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Oxidative DNA damage and its repair in primary rat hepatocyte cultures was investigated following 4 h of incubation with the toxic iron chelate, ferric nitrilotriacetate (Fe-NTA), in the presence or absence of the potent protective flavonoid myricetin (25-50-100 microM). Seven DNA base oxidation products were quantified in DNA extracts by gas chromatography-mass spectrometry (GC-MS) in selected ion monitoring mode. Concomitantly, DNA repair capacity of hepatocytes was estimated by the release of oxidized-base products into culture media, using the same GC-MS method. A genotoxic effect of Fe-NTA (100 microM) in hepatocytes was evidenced by a severe increase in DNA oxidation over basal levels, with accumulation in cellular DNA of five oxidation products derived from both purines and pyrimidines. This prooxidant effect of iron was also noted by an induction of lipid peroxidation, estimated by free malondialdehyde production. Addition of increasing concentrations of myricetin (25-50-100 microM) simultaneously with iron prevented both lipid peroxidation and accumulation of oxidation products in DNA. Moreover, as an activation of DNA repair pathways, myricetin stimulated the release of DNA oxidation bases into culture media, especially of purine-derived oxidation products. This removal of highly mutagenic oxidation products from DNA of hepatocytes might correspond to an activation of DNA excision-repair enzymes by myricetin. This was verified by RNA blot analysis of DNA polymerase beta gene expression which was induced by myricetin in a dose-dependent manner. This represented a novel and original mechanism of cytoprotection by myricetin against iron-induced genotoxicity via stimulation of DNA repair processes. Since iron-induced DNA damage and inefficient repair in hepatocytes could be to genotoxicity and most probably to hepatocarcinogenesis, modulation of these processes in vitro by myricetin might be relevant in further prevention of liver cancer derived from iron overload pathologies.

73. Eur J Nutr 1999 Feb;38(1):28-34

Dietary flavonoids protect human colonocyte DNA from oxidative attack in vitro.

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BACKGROUND & AIMS: Epidemiological studies suggest that antioxidant polyphenols in the human diet may protect against diseases such as cancer. In this study we investigated the cytoprotective potential of the flavonoids, quercetin, myricetin, kaempferol and rutin against oxidative DNA damage in human colonocytes in vitro. METHODS: Caco-2

cells, which display specialised enterocyte/colonocyte cell functions, were used as an in vitro model for human colonocytes. Hydrogen peroxide was employed as the oxidant. DNA damage (strand breakage, oxidised purines and oxidised pyrimidines) was determined using the alkaline single cell gel electrophoresis or comet assay. Cell growth and viability were measured. RESULTS: Hydrogen peroxide caused a dose-dependent increase in DNA strand breakage in human colonocytes, presumably via oxygen free radical generation. Quercetin and myricetin protected Caco-2 cells against oxidative attack. In addition, quercetin decreased hydrogen peroxide-mediated inhibition of growth. Neither rutin nor kaempferol was effective. However, quercetin, while inhibiting DNA strand breakage, did not alter the levels of oxidised bases following peroxide treatment. The antifungal agent ketoconazole, prevented quercetin cytoprotection in Caco-2 cells, indicating that P450-mediated metabolism may alter the efficacy of the flavonoids against oxidative DNA damage. CONCLUSION: Flavonoids, particularly quercetin, the most abundant flavonoid in the human diet, are likely to be important in defending human colonocytes from oxidative attack.

74. Inflamm Res 1998 Nov;47(11):421-7

Anti-inflammatory activity of myricetin-3-O-beta-D-glucuronide and compounds.

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OBJECTIVE AND DESIGN: The anti-inflammatory effect of myricetinglucuronide (MGL) was investigated and structurally-compounds were compared to examine the structure/activity-relationship in carrageenan-induced rat paw edema. MATERIALS AND SUBJECTS: In vitro studies were performed using rat basophilic leukemia (RBL-1) cells, human polymorphonuclear leukocytes (PMNL), COX-1 from ram seminal vesicle, COX-2 from sheep placenta and human venous blood. For the in vivo tests male Wistar rats were used, for the ex vivo test perfused rabbit ears. TREATMENT: 1-300 microg/kg MGL or myricetinmethylglucuronate and 0.1-5 mg/kg other compounds administered p.o. (carrageenan edema). 5, 50 and 150 microg/kg MGL p.o. for 14 days (Freund's adjuvant arthritis), 5 and 50 microg/kg p.o. for 6 days (ulceration). METHODS: Anti-inflammatory effects were measured in carrageenan edema and in adjuvant arthritis. Incidence of gastric lesions was tested in an ulcerogenicity model in vivo. Influence on COX was determined in the perfused rabbit ear, in PMNL and in a test assay

using COX-1 and COX-2. 5-LOX activity was studied using PMNL and RBL-1. The influence on platelet aggregation was evaluated measuring light transmission. RESULTS: MGL exerted a marked and dose-dependent anti-inflammatory effect in acute (carrageenan edema, ED50 15 microg/kg, indomethacin ED50 10 mg/kg) and chronic (adjuvant arthritis, inhibition at 150 microg/kg 18.1 % left paw, 20.6% right paw, indomethacin 3 mg/kg 18.0% and 19.4%)) models of inflammation. In the perfused rabbit ear 1 microg MGL inhibited the release of PGI2, PGD2 and PGE2 to the same extent as 1 microg indomethacin. The inhibition of COX-1 in the intact cell system was IC50 = 0.5 microM, that of indomethacin 0.0038 microM. In the isolated enzyme preparations of COX-1 and COX-2 the IC50 was 10 microM and 8 microM, that of indomethacin 9.2 mM and 2.4 microM. In the RBL-1 and PMNL test assay the inhibition of 5-LOX was 0.1 microM and 2.2 microM. An orally administered dose of 50 microg/kg/day induced no gastric ulcers in rats treated for 6 days. The investigations on carrageenan edema showed a close relationship between the structure of MGL and the anti-inflammatory effect. CONCLUSIONS: MGL is a COX-1, COX-2 and 5-LOX inhibitor. In view of the moderate in vitro activity and the very potent in vivo activity an additive mechanism must be involved. Small changes in the molecular structure lead to the loss or reduction of the anti-inflammatory activity.

75. Mutat Res 1997 Oct 24;393(3):223-31

Quercetin and myricetin protect against hydrogen peroxide-induced DNA damage (strand breaks and oxidised pyrimidines) in human lymphocytes.

Duthie SJ, Collins AR, Duthie GG, Dobson VL
Rowett Research Institute, Bucksburn, Aberdeen, Scotland, UK.
sd@rri.sari.ac.uk

The effects of the flavonoids quercetin and myricetin, and the antihepatotoxic agent silymarin, on hydrogen peroxide-mediated DNA damage in human lymphocytes were determined using alkaline single-cell gel electrophoresis (the comet assay). Treatment with hydrogen peroxide increased the levels of DNA strand breaks and oxidised pyrimidine bases in these cells. Quercetin was protective at concentrations above 10 microM and myricetin decreased oxidant-induced DNA strand breakage at concentrations of 100 microM. Cellular metabolism may alter the antioxidant efficacy of the flavonoids. Silymarin had no protective effect at any of the concentrations tested. None of these flavonoids was itself genotoxic. Neither alpha-tocopherol nor beta-carotene decreased

hydrogen peroxide-induced DNA breakage. The differences in effectiveness of these dietary compounds against oxidative DNA damage may be explained by differences in their chemical structure or location within the cell.

76. Gen Pharmacol 1997 Aug;29(2):121-6

Biological effects of myricetin.

Ong KC, Khoo HE

Department of Biochemistry, Faculty of Medicine, National University of Singapore, Kent Ridge Crescent, Singapore.

1. Myricetin is a natural bioflavonoid whose occurrence in nature is widespread among plants. 2. It has been demonstrated to possess both antioxidative properties and prooxidative properties. 3. It is a potent anticarcinogen and antimutagen, although it has been shown to promote mutagenesis with the use of the Ames Test. 4. Its therapeutic potential and benefits in cardiovascular diseases and diabetes mellitus also are reviewed.

77. J Pharm Pharmacol 2000 Apr;52(4):451-9

Identification of kaempferol as a monoamine oxidase inhibitor and potential Neuroprotectant in extracts of Ginkgo biloba leaves.

Sloley BD, Urichuk LJ, Morley P, Durkin J, Shan JJ, Pang PK, Coutts RT

CV Technologies, Edmonton, Alberta, Canada.

The effects of Ginkgo biloba leaf extract on rat brain or livermonoamine oxidase (MAO)-A and -B activity, biogenic amine concentration in nervous tissue, N-methyl-D-aspartate (NMDA)- and N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4)-induced neurotoxicity and antioxidant activity was investigated to determine the effects of the extract on monoamine catabolism and neuroprotection. Ginkgo biloba leaf extract was shown to produce in-vitro inhibition of rat brain MAO-A and -B. The Ginkgo biloba extract was chromatographed on a reverse-phase HPLC system and two of the components isolated were shown to be MAO inhibitors (MAOIs). These MAOIs were identified by high-resolution mass spectrometry as kaempferol and isorhamnetin. Pure kaempferol and a number of flavonoids were examined as MAOIs in-vitro. Kaempferol, apigenin and chrysin proved to be potent MAOIs, but produced more pronounced inhibition of MAO-A than MAO-B. IC50 (50%

inhibition concentration) values for the ability of these three flavones to inhibit MAO-A were 7 x 10(-7), 1 x 10(-6) and 2 x 10(-6) M, respectively. Ginkgo biloba leaf extract and kaempferol were found to have no effect ex-vivo on rat or mouse brain MAO or on concentrations of dopamine, noradrenaline, 5-hydroxytryptamine and 5-hydroxyindoleacetic acid. Kaempferol was shown to protect against NMDA-induced neuronal toxicity in-vitro in rat cortical cultures, but did not prevent DSP-4-induced noradrenergic neurotoxicity in an in-vivo model. Both Ginkgo biloba extract and kaempferol were demonstrated to be antioxidants in a lipid-peroxidation assay. This data indicates that the MAO-inhibiting activity of Ginkgo biloba extract is primarily due to the presence of kaempferol. Ginkgo biloba extract has properties indicative of potential neuroprotective ability.

78. J Neurosci Res 1999 Aug 1;57(3):399-404

Phytoestrogen kaempferol (3,4',5,7-tetrahydroxyflavone) protects PC12 and T47D cells from beta-amyloid-induced toxicity.

Roth A, Schaffner W, Hertel C

F. Hoffmann-LaRoche Ltd, Pharma Research Preclinical, Basel, Switzerland.

In clinical studies, it has been shown that estrogen replacement therapy in menopause is strongly cor with a reduced risk of the development of Alzheimer's disease (AD). In in vitro experiments, it was demonstrated that estradiol protects cells against the toxic effects of beta-amyloid, the major component of plaques in brains of AD patients. Therefore, estrogens have become interesting candidates for a possible treatment of neurodegeneration. In plants, a class of compounds has been identified that bind to human estrogen receptor, so-called phytoestrogens, which are part of our daily diet. Here, we compared the effects of alpha- and beta-estradiol with plant-derived kaempferol on beta-amyloid peptide-induced toxicity in PC12 neuroblastoma and T47D human breast cancer cells. The present results demonstrate a protective effect of kaempferol comparable to that observed with estradiol. The effects of the weak estrogen receptor agonists alpha-estradiol and kaempferol were found to be similar to the effects of the strong estrogen receptor agonist beta-estradiol, suggesting a mode of action independent from the nuclear estrogen receptor.

79. Arch Pharm Res 1994 Apr;17(2):71-5

Antimutagenic effect of plant flavonoids in the Salmonella assay system.

Choi JS, Park KY, Moon SH, Rhee SH, Young HS
Dept. of Food and Nutrition, National Fisheries University of Pusan, Korea.

The antimutagenic effects of 27 kinds of plant flavonoids on the mutagenicity of aflatoxin B1(AFB1) and N-methyl-N'-nitro-N-nitrosoguanidine(MNNG) in Salmonella typhimurium TA100 were investigated. In the mixed applications of AFB1 (1 microgram/plate) with the flavonoids (300 micrograms/plate) in the presence of a mammalian metabolic activation system (S9 mix), chrysin, apigenin, luteolin and its glucoside, kaempferol, fisetin, morin, naringenin, hesperetin, persicogenin, (+)-catechin and (-)-epicatechin showed the antimutagenic effect against AFB1 with more than 70% inhibition rate. A little or no antimutagenicities except flavone against MNNG (0.5 microgram/plate) were observed. For the antimutagenicity of the flavonoids on AFB1, the flavonoid structure that contains the free 5-, 7-hydroxyl group seemed to be essential. However, saturation of the 2,3-double bond or elimination of the 4-keto group did
not affect the activity.

80. Nutr Cancer 1999;34(2):160-6

Protection by the flavonoids myricetin, quercetin, and rutin against hydrogen peroxide-induced DNA damage in Caco-2 and Hep G2 cells.

Aherne SA, O'Brien NM
Department of Food Science and Technology, University College, Cork, Republic of Ireland.

Flavonoids are reported to exhibit a wide variety of biological effects, including antioxidant and free radical-scavenging activities. Reactive oxygen species have been implicated in a range of human pathological diseases such as atherosclerosis and certain cancers. The aims of this present study were 1) to investigate the effect of the flavonoids myricetin, quercetin, and rutin on cell viability, endogenous antioxidant enzyme activities, and DNA integrity in Caco-2 and Hep G2 cells and 2) to determine whether these flavonoids could protect against H2O2-induced DNA damage. Both cell lines were supplemented with various concentrations (0-200 microM) of myricetin, quercetin, and rutin for 24 hours or H2O2 (50 microM) for 30 minutes, and cell viability was assessed. Over the concentration range tested, neither the flavonoids nor H2O2 significantly affected cell viability. The effect of the flavonoids on the activities of the antioxidant enzymes catalase (EC 1.11.1.6) and superoxide dismutase (EC 1.15.1.1) and on DNA

integrity was assessed. The flavonoids did not significantly affect catalase or superoxide dismutase activity and did not induce DNA damage in either cell line. Exposure to 50 microM H2O2 for 30 minutes at 37 degrees C resulted in significant DNA damage, and preincubation with the flavonoids before H2O2 exposure significantly ($p < 0.05$) protected Caco-2 and Hep G2 cells against H2O2-induced DNA damage.

81. Nutr Cancer 1999;34(1):88-99t

Quercetin-induced apoptosis in colorectal tumor cells: possible role of EGF receptor signaling.

Richter M, Ebermann R, Marian B
Institute for Tumor Biology-Cancer Research, University of Vienna, Austria.

Flavonoids are among the best candidates for mediating the protective effect of diets rich in fruits and vegetables with respect to colorectal cancer. To gain additional information about their growth effects on colorectal tumors and their cellular mechanisms of action, a series of flavonoids was added to cultures of colonic tumor cells. Most compounds induced growth inhibition and cell loss at concentrations of 1-100 microM, relative effectivity being quercetin > apigenin > fisetin > robinetin and kaempferol. Myricetin was only slightly effective. Quercetin was the strongest inducer of apoptosis in a process that was reversible until 10 hours by flavonoid removal and until 24 hours by fetal calf serum. Cells were preferentially retained in the S phase. On the cellular level, quercetin sensitivity was cor with epidermal growth factor (EGF) receptor levels, rapid growth, and poor differentiation, indicating the possibility of targeting those cells most harmful for the organism. The flavonoid transiently inhibited EGF receptor phosphorylation but had only little effect on other signaling molecules. Even after recovery of receptor phosphorylation, cells remained resistant to EGF stimulation. In summary, the data indicate that inhibition of EGF receptor kinase is an integral part of quercetin-induced growth inhibition, but additional mechanisms also contribute to the overall effect.

4.3.C References on Povidone and Crospovidone from Toxline Database

The following references include everything published concerning the toxicity of PVPP and PVP from 1924 to April 1999. It is interesting to note that this database lists PVP and PVPP as synonomous. There is no data presented in these references which indicates that PVPP is unsafe as a food additive in the amounts proposed.

104881DIALOG(R)File 336:RTECSComp & dist by NIOSH, Intl Copyright All Rights Res. All rights reserved.

104881
RTECS Number: TR8370000

Substance Name: Poly(1-vinyl-2-pyrrolidinone) homopolymer
CAS Registry Number: 9003-39-8
Molecular Formula: (C6H9NO)x
Synonyms: Agent AT 717 ; Agrimer ; Albigen A ; Aldacol Q ; AT 717 ; Bolinan ; 1-Ethenyl-2-pyrrolidinone polymers ; Ganex P 804 ; Hemodesis ; Hemodez ; K15 ; K25 ; K25 (polymer) ; K30 ; K30 (polymer) ; K60 ; K60 (polymer) ; K90 ; K115 ; K115 (polyamide) ; Kollidon ; Kollidon 17 ; Kollidon 25 ; Kollidon 30 ; Luviskol ; Luviskol K30 ; Luviskol K90 ; MPK 90 ; NCI C60582 ; Neocompensan ; Peragal ST ; Peregal ST ; Periston ; Periston-N ; Peviston ; Plasdone ; Plasdone K-26/28 ; Plasdone K 29-32 ; Plasdone XL ; Plasmosan ; Polyclar AT ; Polyclar H ; Polyclar L ; Poly(1-(2-oxo-1-pyrrolidinyl)ethylene) ; Polyvidone ; Poly(n-vinylbutyrolactam) ; Poly(vinylpyrrolidinone) ; Poly(N-vinylpyrrolidinone) ; Poly(1-vinylpyrrolidinone) ; Polyvinylpyrrolidone ; Povidone ; Povidone (usp xix) ; Protagent ; PVP ; PVP 40 ; PVP-K 15 ; PVP-K 30 ; PVP-K 60 ; PVP-K 90 ; PVPP ; 2-Pyrrolidinone, 1-ethenyl, homopolymer ; 2-Pyrrolidinone, 1-vinyl-, polymers ; 143 RP ; Subtosan ; Vinisil ; N-Vinylbutyrolactam polymer ; Vinylpyrrolidinone polymer ; N-Vinylpyrrolidinone polymer ; N-Vinylpyrrolidone polymer ; Vinylpyrrolidone polymer
Compound Class: Tumorigen
Wiswesser Line Notation: /T5NVTJ AY*1*/
Record Date: 199710

TOXICITY EFFECTS DATA:
Hypermotility, diarrheaOralRatLD50 100gm/kgFAONAU
53A,486,1974
* OralMouseLD50 >40gm/kgFAONAU40,164,1967
* IntraperitonealMouseLD50 12gm/kgFAONAU53A,486,1974
* UnreportedMouseLD50 16gm/kgKHFZAN19,212,1985
* OralRabbitLD50 1040mg/kgVETNAL61(12),68,1985
Hypermotility, diarrheaOralGuinea pigLD50 100gm/kgFAONAU
53A,486,1974

REVIEWS

IARC Cancer Review:Group 3 IMSUDL 7,56,1987
IARC Cancer Review:Animal Limited Evidence IMEMDT 19,461,1979
IARC Cancer Review:Human No Adequate Data IMEMDT 19,461,1979
TOXICOLOGY REVIEW FAONAU 53A,486,1974
TOXICOLOGY REVIEW JJOMDZ 15,808,1973
TOXICOLOGY REVIEW ARZNAD 21,1468,1971
TOXICOLOGY REVIEW 15SWA8 21,427,1970
TOXICOLOGY REVIEW PSTGAW 38,1,1962
TOXICOLOGY REVIEW PSTGAW 38,1,1962
TOXICOLOGY REVIEW JSCCA5 5,129,1954

NIOSH CRITERIA DOCUMENTS:
NOHS 1974: HZD 81235; NIS 50; TNF 7856; NOS 63; TNE 102100
NOES 1983: HZD X7211; NIS 46; TNF 7688; NOS 63; TNE 161259; TFE 84819

NTP, NIOSH, EPA STATUS:
EPA TSCA Section 8(b) CHEMICAL INVENTORY
EPA TSCA Section 8(d) unpublished health/safety studies
EPA TSCA TEST SUBMISSION (TSCATS) DATA BASE, DECEMBER 1998

TOXICITY EFFECTS JOURNAL REFERENCES:
FAONAU FAO Nutrition Meetings Report Series.Rome, Italy No.?-57,
1948-77. Discontinued.

KHFZAN Khimiko-Farmatsevticheskii Zhurnal. Chemical Pharmaceutical
Journal. For English translation, see PCJOAU.V/O Mezhdunarodnaya

Kniga, 113095 Moscow, USSR V.1- 1967-

VETNAL Veterinariya. Veterinary Science.V/O Mezhdunarodnaya Kniga, 113095 Moscow, USSR V.1- 1924-

REVIEWS JOURNAL REFERENCES:

ARZNAD Arzneimittel-Forschung. Drug Research.Editio Cantor Verlag, Postfach 1255, W-7960 Aulendorf, Fed. Rep. Ger. V.1- 1951-

FAONAU FAO Nutrition Meetings Report Series.Rome, Italy No.?-57, 1948-77. Discontinued.

IMEMDT IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man.WHO Publications Centre USA, 49 Sheridan Ave., Albany, NY 12210 V.1- 1972-

IMSUDL IARC Monographs, Supplement.WHO Publications Centre USA, 49 Sheridan Ave., Albany, NY 12210 No.1- 1979-

JJOMDZ JOM, Journal of Occupational Medicine.American Occupational Medicine Assoc., 150 N. Wacker Dr., Chicago, IL 60606 V.10- 1968-

JSCCA5 Journal of the Society of Cosmetic Chemists.Soc. of Cosmetic Chemists, 1995 Broadway, Suite 1701, New York, NY 10023 V.1- 1947-

PSTGAW Proceedings of the Scientific Section of the Toilet Goods Association.Washington, DC No.1-48, 1944-67. Discontinued.

15SWA8 Kirk-Othmer Encyclopedia of Chemical Technology, 1963-70,2nd ed., Standen, A., et al., eds., New York, John Wiley & Sons, Inc., 1971

DATA PRESENT: Toxicity Effects; Reviews; NIOSH Criteria Documents; NTP, NIOSH, EPA Status

4.4 Adverse effects and/or consumer complaints

ShanStar Cranberry extract is being taken by several persons on a voluntary basis. They have been ingesting 1 to 6 grams daily for periods of up to 22 months. The product is administered in beverages, yogurt, ice cream and on cereals. To date there are no adverse effects or complaints.

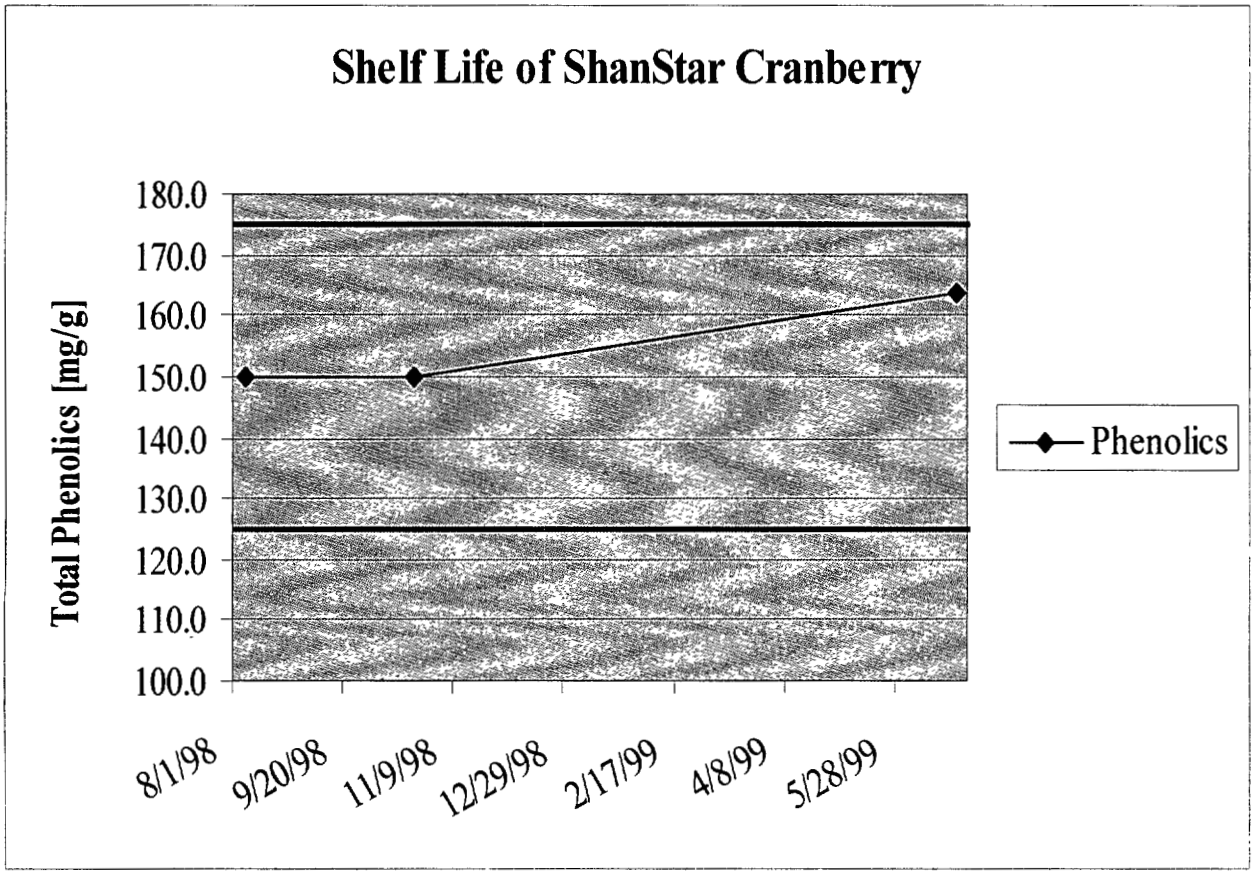
4.5 Stability data and proposed expiration date

4.5.A Stability data

The batch being repeated ly tested for stability was manufactured 8/7/1998.

Test results:

Date	Total Phenolics
8/7/1998	150 +/- 25 mg/g
10/23/1998	149.9 +/- 25 mg/g
6/25/1999	163.6 +/- 25 mg/g



000505

4.5.B Expiration Date

The expiration date of ShanStar cranberry is 2 years. This expiration date is subject to change as future test results are produced.

The expiration date is based on current data in Section 4.5.A, an accelerated aging test at high temperatures and on knowledge of the stabilizing properties of PVPP and the anti-oxidant properties of plant phenolics.

4.6 MSDS for ShanStar Cranberry Extract

See MSDS from Cliffstar Corporation in following pages.

MATERIAL SAFETY DATA SHEET

MSDS: 0001

Date: 2/26/99
24-HOUR EMERGENCY TELEPHONE: (CHEMTREC)
(800) 424-9300

MANUFACTURER'S NAME: Cliffstar Corporation
MANUFACTURER'S ADDRESS: 1 Cliffstar Avenue
Dunkirk, New York 14048
INFORMATION TELEPHONE NUMBER: (716) 366-6100

SECTION 01: PRODUCT IDENTIFICATION

PRODUCT NAME: Shanstar Cranberry **CLIFFSTAR ITEM NUMBER:**

FORMULA NAME: Specific formulation held as a trade secret.
SYNONYM: N/A
CAS: N/A

SECTION 02: PHYSICAL AND CHEMICAL CHARACTERISTICS

Appearance: Dark red to magenta powder.
Odor: Fruity cranberry.
Flavor: Bitter, astringent.
Specific Gravity: No data found.
Boiling Point: No data found.
Vapor Pressure: Not volatile.
Vapor Density: Not volatile.
Water Solubility: Insoluble.
Melting/Freezing Point: No data found.

SECTION 03: COMPOSITION / INFORMATION ON HARZARDOUS INGREDIENTS

None

000508

000508

[redacted]

SECTION 04: HAZARDS IDENTIFICATION

**********EMERGENCY OVERVIEW**********

Free flowing. Dark red to magenta powder.
No known hazards.

****

Nuisance Dust Limits:

Permissible Exposure Limit (PEL): 15MG/M3 – total; 5MG/MG - respirable
Threshold Limit Value (TLV): TWA 10MG/M3 - total; 5 MG/M3 - respirable

Health Hazard Determination: None of the ingredients have been listed as a carcinogen by NTP (National Toxicology Program), OSHA (Occupational Safety & Health Administration), or IARC (International Agency for Research on Cancer). The mixture may cause the following upon contact:

Skin/Eyes: May possibly cause irritation in some individuals upon prolonged contact.
Ingestion: No known health hazards.
Inhalation: No known health hazards.
Misc.: No known health hazards.
Medical Conditions Generally Recognized as Being Aggravated by Exposure: None known for normal conditions of use.

SECTION 05: FIRST AID MEASURES

First Aid For Ingestion:
General precautionary measures suggest inducing vomiting immediately by giving two glasses of water and sticking finger down throat. Never give anything by mouth to an unconscious person. Call a physician.

First Aid for Inhalation:
None required.

First Aid for Skin Contact:
As for all foreign materials, wash after exposure.

First Aid for Eye Contact:
As for all foreign materials, immediately flush eyes with plenty of water.

000509 [redacted]

SECTION 06: FIREFIGHTING MEASURES

Flash Point: No flash point.
Autoignition Temperature: No data found.
Flammable Limits: No data found.
Fire Fighting Media: Use media proper to primary cause if fire.
Special Fire Fighting Procedures: None known.
Fire/Explosion Hazards: None known.

NFPA Hazard Codes – Health / Flammability / Reactivity	**1**	**0**	**1**
HMIS Hazard Codes – Health / Flammability / Reactivity	**1**	**0**	**1**

SECTION 07: STABILITY AND REACTIVITY

Stability: Stable.
Hazardous Polymerization: Will not occur.
Conditions to Avoid: None known.

Incompatible Materials: Strong oxidizing or reducing agents.
Hazardous Decomposition Products: Toxic fumes of NOx emitted when heated to decomposition.

SECTION 08: ACCIDENTAL RELEASE MEASURES

Spill / Leak Clean-up Procedures: Scoop up, avoid dusting. Dispose of solid waste according to local, state, and federal regulations. Flush spill area with water.

EPA Hazardous Substance Reportable Quantity: Not listed.

SECTION 09: HANDLING AND STORAGE

Precautionary Measures: Avoid breathing dust.
Disposal Method: Dispose of with solid waste according to local, state, and federal regulations.

[redacted]

RCRA Class: Not regulated.

SECTION 10: EXPOSURE CONTROLS/PERSONAL PROTECTION

Ventilation: General precautionary measures suggest use with adequate ventilation.
Respiratory Protection: Dust mask where dusting cannot be avoided.
Eye Protection: safety glasses.
Skin Protection: None required.
Personal Hygiene: Observe ordinary measures of personal hygiene.

Protective Measures to Undertake for Repair/Maintenance of Equipment:
Wash equipment thoroughly with steam or warm water until clean. Check for flammables with an explosion meter, and check the oxygen level with an oxygen meter. In all cases, follow good industrial safety practices before entering equipment.

Nuisance Dust Limits:
Permissible Exposure Limit (PEL): 15MG/M3 – total; 5MG/MG - respirable
Threshold Limit Value (TLV): TWA 10MG/M3 - total; 5 MG/M3 - respirable

SECTION 11: TRANSPORT INFORMATION

Domestic Data:
DOT Shipping Name: Not regulated.
DOT Hazard Class: Not regulated.
Hazardous Ingredients: None.
UN Number: None.

Disclaimer: The information contained herein is based on data considered to be accurate and reliable. No warranty is expressed or implied regarding the accuracy or correctness of this data. It is the user's obligation to determine the safe use of the product since conditions of use, handling, storage, and disposal are beyond our control.

4.7 MSDS for Polyplasdone Crospovidone NF (Polyvinylpolyyrrolidone)

See MSDS from ISP in following pages.

[redacted]

000512

[redacted]

ISP TECHNOLOGIES INC.
201 628 4000
1361 Alps Road
Wayne, NJ 07470
Medical Information: 612 221 3999 Ext. 016
Transportation Emergency: CHEMTREC 1 800 424 9300

MATERIAL SAFETY DATA SHEET

POLYPLASDONE XL

SECTION 01 - CHEMICAL PRODUCT AND COMPANY IDENTIFICATION

DOMESTIC TRADE NAME: **POLYPLASDONE XL**
EXPORT TRADE NAME: POLYPLASDONE XL

CAS REGISTRY NO.: 0009003-39-8
CAS REGISTRY NAME: 2-PYRROLIDINONE, 1-ETHENYL-, HOMOPOLYMER
SYNONYM: POLY(VINYLPOLYPYRROLIDONE)

MOLECULAR FORMULA: $(C_6H_9NO)_x$

SECTION 02 - COMPOSITION/INFORMATION ON HAZARDOUS INGREDIENTS

NONE

SECTION 03 - HAZARDS IDENTIFICATION

```
************ EMERGENCY OVERVIEW ************
*                                          *
* FREE FLOWING, WHITE TO OFF-WHITE POWDER. *
* NO KNOWN HAZARDS.                        *
*                                          *
********************************************
```

-ACUTE TOXICITY-

ORAL TOXICITY:
RAT LD50 > 100,000 MG/KG

DERMAL TOXICITY:
NOT ABSORBED TOPICALLY

INHALATION TOXICITY:
HUMAN, GUINEA PIG AND RABBIT; EXPOSURE TO AEROSOLS AND DUSTS OF SMALL PARTICLE SIZE PRODUCED NO EVIDENCE OF HARMFUL EFFECTS.

SKIN IRRITATION:
HUMAN REPEAT INSULT PATCH TEST; NOT A PRIMARY IRRITANT.

[redacted] 000513

ISP TECHNOLOGIES INC.
201 628 4000
1361 Alps Road
Wayne, NJ 07470
Medical Information: 612 221 3999 Ext. 016
Transportation Emergency: CHEMTREC 1 800 424 9300

SECTION 03 - HAZARDS IDENTIFICATION (CONT.)

EYE IRRITATION:
RABBIT; NO IRRITATION OBSERVED.

SENSITIZER: NO
HUMAN REPEAT INSULT PATCH TEST; NOT A SENSITIZER.

DOT CORROSIVE:
NO DATA FOUND

PRIMARY ROUTE(S) OF ENTRY: CONTACT

-SIGNS AND SYMPTOMS OF EXPOSURE-

SYMPTOMS OF INGESTION:
NO EFFECTS OF EXPOSURE EXPECTED.

SYMPTOMS OF INHALATION:
NO EFFECTS OF EXPOSURE EXPECTED.

SYMPTOMS OF SKIN CONTACT:
NO EFFECTS OF EXPOSURE EXPECTED DUE TO ABSORPTION OR CONTACT. MAY POSSIBLY CAUSE IRRITATION OR DERMATITIS IN SOME INDIVIDUALS UPON PROLONGED CONTACT.

SYMPTOMS OF EYE CONTACT:
NO EFFECTS OF EXPOSURE EXPECTED WITH THE EXCEPTION OF POSSIBLE IRRITATION.

MEDICAL CONDITIONS AGGRAVATED BY EXPOSURE:
NO DATA FOUND

-OTHER TOXICITY-

OTHER ACUTE TOXICITY:
NO DATA FOUND

SUB CHRONIC ORAL TOXICITY:
RODENT AND DOG STUDIES; NO ADVERSE EFFECT LEVEL EXCEEDS 10% OF TOTAL DIET; NO EVIDENCE OF CARCINOGENIC ACTIVITY.

SUB CHRONIC INHALATION TOXICITY:
NO DATA FOUND

SUB CHRONIC MISCELLANEOUS TOXICITY:
NO DATA FOUND

AQUATIC TOXICITY:
NO DATA FOUND

[redacted]

ISP TECHNOLOGIES INC.
201 628 4000
1361 Alps Road
Wayne, NJ 07470
Medical Information: 612 221 3999 Ext. 016
Transportation Emergency: CHEMTREC 1 800 424 9300

SECTION 03 - HAZARDS IDENTIFICATION (CONT.)

TUMORIGENICITY:
CHRONIC RODENT AND DOG FEEDING STUDIES SHOW THAT THE NO ADVERSE EFFECT LEVEL EXCEEDS 10% OF THE DIET WITH NO EVIDENCE OF CARCINOGENIC ACTIVITY.

MUTAGENICITY:
PVP WAS TESTED IN THE AMES ASSAY; DOMINANT LETHAL TEST ON MICE; L5178Y MOUSE (TK +/-) LYMPHOMA ASSAY; BONE MARROW CHROMOSOMAL ABERRATION ASSAY; AND THE BALB/C 3T3 TRANSFORMATION. IN EACH CASE PVP WAS FOUND NOT TO BE MUTAGENIC.

REPRODUCTIVE TOXICITY:
TERATOLOGY STUDIES WITH PVP CONCENTRATIONS UP TO 10% OF THE DIET SHOW NO EMBRYOTOXICITY, FETOTOXICITY OR TERATOGENICITY.

MISCELLANEOUS TOXICITY:
NO DATA FOUND

SECTION 04 - FIRST AID MEASURES

FIRST AID FOR INGESTION:
GENERAL PRECAUTIONARY MEASURES SUGGEST INDUCING VOMITING IMMEDIATELY BY GIVING TWO GLASSES OF WATER AND STICKING FINGER DOWN THROAT. NEVER GIVE ANYTHING BY MOUTH TO AN UNCONSCIOUS PERSON. CALL A PHYSICIAN.

FIRST AID FOR INHALATION:
NONE REQUIRED.

FIRST AID FOR SKIN CONTACT:
FOR ALL FOREIGN MATERIALS, WASH AFTER EXPOSURE.

FIRST AID FOR EYE CONTACT:
FOR ALL FOREIGN MATERIALS, IMMEDIATELY FLUSH EYES WITH PLENTY OF WATER.

SECTION 05 - FIREFIGHTING MEASURES

FLASH POINT: NO FLASH POINT
AUTOIGNITION TEMP: NO DATA FOUND
FLAMMABLE LIMITS: NO DATA FOUND
FIRE FIGHTING MEDIA:
USE MEDIA PROPER TO PRIMARY CAUSE OF FIRE.
SPECIAL FIRE FIGHTING PROCEDURES:
NONE KNOWN

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SECTION 05 - FIREFIGHTING MEASURES (CONT.)

FIRE/EXPLOSION HAZARDS:
NONE KNOWN

NFPA HAZARD CODES - HEALTH/FLAMMABILITY/REACTIVITY	1	0	0
HMIS HAZARD CODES - HEALTH/FLAMMABILITY/REACTIVITY	0	0	0

SECTION 06 - ACCIDENTAL RELEASE MEASURES

SPILL/LEAK CLEAN-UP PROCEDURES:
SCOOP UP. AVOID DUSTING. DISPOSE OF WITHSOLID WASTE ACCORDING TO FEDERAL, STATE AND LOCAL REGULATIONS. FLUSH SPILL AREA WITH WATER.

EPA HAZARDOUS SUBSTANCE REPORTABLE QUANTITY: NOT LISTED

SECTION 07 - HANDLING AND STORAGE

PRECAUTIONARY MEASURES:
AVOID BREATHING DUST.

DISPOSAL METHOD:
DISPOSE OF WITH SOLID WASTE ACCORDING TO FEDERAL, STATE AND LOCAL REGULATIONS.

RCRA CLASS: NOT REGULATED

SECTION 08 - EXPOSURE CONTROLS/PERSONAL PROTECTION

VENTILATION:
GENERAL PRECAUTIONARY MEASURES SUGGEST USE WITH ADEQUATE VENTILATION.

RESPIRATORY PROTECTION:
DUST MASK WHERE DUSTING CANNOT BE AVOIDED.

EYE PROTECTION:
SAFETY GLASSES.

SKIN PROTECTION:
NONE REQUIRED.

PERSONAL HYGIENE:
OBSERVE ORDINARY MEASURES OF PERSONAL HYGIENE.

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SECTION 08 - EXPOSURE CONTROLS/PERSONAL PROTECTION (CONT.)

PROTECTIVE MEASURES DURING REPAIR/MAINTENANCE OF EQUIPMENT:
WASH EQUIPMENT THOROUGHLY WITH STEAM OR WARM WATER UNTIL CLEAN. CHECK FOR FLAMMABLES WITH AN 'EXPLOSION METER' AND ALSO CHECK THE OXYGEN LEVEL WITH AN OXYGEN METER. IN ALL CASES, FOLLOW GOOD INDUSTRIAL SAFETY PRACTICES BEFORE ENTERING EQUIPMENT.

EXPOSURE LIMITS:
NUISANCE DUST: TLV:TWA 10 MG/M3-TOTAL; 5 MG/M3-RESPIRABLE.
PEL:TWA 15 MG/M3-TOTAL; 5 MG/M3-RESPIRABLE.

SECTION 09 - PHYSICAL AND CHEMICAL PROPERTIES

BOILING POINT: NO DATA FOUND
VAPOR PRESSURE: NOT VOLATILE
VAPOR DENSITY (AIR=1): NOT VOLATILE
WATER SOLUBILITY: INSOLUBLE
MELTING/FREEZING POINT: NO DATA FOUND
APPEARANCE: WHITE TO OFF-WHITE FREE FLOWING POWDER
SPECIFIC GRAV. (WATER=1): NO DATA FOUND
PERCENTAGE VOLATILES: 5.00 % WATER-MAX
EVAPORATION RATE: NOT VOLATILE
pH OF SOLUTION: 5.00 - 8.00 1 G/100 ML WATER
ODOR: NO DATA FOUND

SECTION 10 - STABILITY AND REACTIVITY

STABILITY: STABLE
HAZARDOUS POLYMERIZATION: WILL NOT OCCUR
CONDITIONS TO AVOID:
NONE KNOWN

INCOMPATIBLE MATERIALS:
STRONG OXIDIZING OR REDUCING AGENTS.

HAZARDOUS DECOMPOSITION PRODUCTS:
TOXIC FUMES OF NOx EMITTED WHEN HEATED TO DECOMPOSITION.

SECTION 11 - TRANSPORT INFORMATION

-DOMESTIC DATA-
DOT SHIPPING NAME: NOT REGULATED

[redacted]

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SECTION 11 - TRANSPORT INFORMATION (CONT.)

DOT HAZARD CLASS: NOT REGULATED
HAZARDOUS INGREDIENT(S): NONE
UN NUMBER: NONE

-EXPORT DATA-
EXPORT SHIPPING NAME: NOT REGULATED
EXPORT HAZARD CLASS: NOT REGULATED
HAZARDOUS INGREDIENT(S): NONE
UN NUMBER: NONE

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4.8 Evaluation of dietary exposure

Based on our estimate of dietary exposure and review of the data listed below, ShanStar Cranberry in beverages, and in foods and beverages for special dietary use is considered GRAS.

Dietary exposure table

Proposed use	Minimum level	Maximum level	Estimated daily exposure
Beverage for special dietary use	None	0.5 mg per ml	240 mg in 480 ml (4 x 120 ml servings)
Food for special dietary use	None	0.5 mg per g	300 mg in 600 g of solid food (3 x 200 g servings)
Regular beverages	None	0.1 mg/ml	150 mg in 1500 ml (4 x 375 ml servings)

The food and beverages for special dietary use will be distributed only to longterm care facilities. The patients consuming these products may have access to other beverages containing ShanStar Cranberry. If we assume they consume a maximum of the beverage for special dietary use---480 ml--- and then another 1020 ml of regular beverage to add up to a daily intake of 1500 ml of beverage, they may consume up to a maximum of 342 mg of ShanStar Cranberry in beverage and 300 mg in food, or a total dietary exposure of 642 mg of ShanStar Cranberry per day.

Those persons outside of longterm care facilities will only have accessibility to regular beverages containing ShanStar Cranberry at a level of 0.1 mg/ml. If we assume that all beverage that they drink contains ShanStar Cranberry, a conservative estimate of their daily exposure would be 150 mg.

Key data, presented during the review, include:

- Data from Drug Master File, Type V, #2297 for Crospovidone.
- Summary of studies on ADI of PVPP cited by JECFA/FAO/WHO; approximately 5 grams per kilogram of bodyweight in mice and dogs showed no adverse effects
- Allowable Daily Intake of Povidone (PVP, Polyvinylpyrrolidone), 50 mg per kg of body weight
- Data proving the equivalency of the polyphenolics in cranberry juice with those found in ShanStar Cranberry extract
- Data from volunteers consuming between 1 to 6 grams of ShanStar Cranberry per day
- Acute Oral toxicity studies of ShanStar Cranberry extract
- Estimated intake of plant phenolics and quercetin, myricetin and kaempferol
- Quantitation of quercetin, myricetin and kaempferol in ShanStar Cranberry.

Section 5. Non-Clinical Laboratory Studies

5.1 In vitro antimicrobial susceptibility assay report and protocol- Natural pH of product

5.1.A Results at natural pH of product

5.1.B Protocol for testing at natural pH

5.2 In vitro antimicrobial susceptibility assay report and protocol - pH 5.0

5.2.A Results at pH 5.0

5.2.B Protocol for testing at pH 5.0

5.3 HPLC Total Phenolic Testing

5.4 Animal Studies

5.4.A Summary of Acute Oral Toxicity of ShanStar Cranberry extract

5.4.B Acute Oral Toxicity of ShanStar Cranberry extract

5.4.C Summary of Studies on Allowable Daily Intake of PVPP

5.5 Pesticide analysis of ShanStar Cranberry extract

5.6 Analysis of ShanStar Cranberry for quercetin

5.7 Estimation of myricetin and kaempferol

5.1. In vitro antimicrobial susceptibility assay reports and protocols

5.1.A Antimicrobial Susceptibility Assay at the natural pH of the product

OBJECTIVE: To evaluate the antimicrobial potency of the Shanstar-cranberry product against *Escherichia coli* and to compare its antimicrobial potency with various other commercially available products. The antimicrobial assay has been developed to assess the extent at which the product can be diluted and still maintain its inhibitory activity.

PROTOCOL: See the attached Antimicrobial Susceptibility Protocol (MIC/MBC).

STRAIN: *Escherichia coli* 25922. This strain is non-pathogenic and recommended by the Association of Official Analytical Chemists (A.O.A.C) for use in most quality control assays.

RESULTS:

Code:

a) **Number**: Number of viable *E.coli* cells remaining after being subjected to a 30 minute incubation period.
b) **TNTC:** Too Numerous To Count
c) + : Bacterial growth present but unable to be accurately counted due to large overlapping colonies.

Conditions:

The number of cells used in each of the dilutions was approximately $1x10^3$ cells per milliliter. The ratio number at the top of table 1 represents the relative amount of test product in saline solution, ending at 1:1024 which is 0.098% of the pure, dry product.

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Results:

The results of this assay reflect the product's potency in terms of its ability to affect ~$1x10^3$ cells of *E. coli.* The dilution sample in which there is less than 0.5% of the original inoculum per $1x10^3$ cells, or 5 colony forming units (CFU), is expressed as the titer. The data shown in table 1 suggests that the titer of the Shanstar - Cranberry is between 1:128 and 1:256. Based on this data, the potency of the Shanstar – Cranberry, expressed in titer, is at least 64 times more potent than most competitors analyzed and 32 times more potent than brand 3. The competitors analyzed by this assay show a very low potency, ranging from zero biological activity and a titer of 1:4.

It is interesting to note that at the titer of 1:256, there is approximately 4.0mg of sample per milliliter of saline solution. This means that 0.4% of the Shanstar - Cranberry product is capable of inhibiting 995 cells by the method employed for this antimicrobial assay.

Table 1. **Shanstar - Cranberry Compared to Several Competitors**
Data represents $1x10^3$cells/mL

Samples	**1:2**	**1:4**	**1:8**	**1:16**	**1:32**	**1:64**	**1:128**	**1:256**	**1:512**	**1:1024**
Shanstar- Cran 1	-	**0**	**0**	**0**	**0**	**0**	**0**	**5**	**>100**	-
Nature's Herbs	**0**	**TNTC**	**TNTC**	**TNTC**	**TNTC**	**TNTC**	**TNTC**	-	-	-
Natural Brands	**0**	**TNTC**	**TNTC**	**TNTC**	**TNTC**	**TNTC**	**TNTC**	-	-	-
Nature's Resources	**0**	**0**	**TNTC**	**TNTC**	**TNTC**	**TNTC**	**TNTC**	-	-	-
Solaray	**0**	**TNTC**	**TNTC**	**TNTC**	**TNTC**	**TNTC**	**TNTC**	-	-	-
Eclectic Institute	**TNTC**	**TNTC**	**TNTC**	**TNTC**	**TNTC**	**TNTC**	**TNTC**	-	-	-
Frontier	**TNTC**	**TNTC**	**TNTC**	**TNTC**	**TNTC**	**TNTC**	**TNTC**	-	-	-
TruNature	**TNTC**	**TNTC**	**TNTC**	**TNTC**	**TNTC**	**TNTC**	**TNTC**	-	-	-

Table 2. **Titer Data of "Plating Supernatant Only" Compared to "Plating Suspended Product"**

Data represents ~$1x10^3$cells/mL

Samples	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024
Shanstar - Cran 1 Supernatant only	-	0	0	0	0	0	0	5	>100	-
Shanstar - Cran 1 Suspended Product	-	0	0	0	0	0	2	28	TNTC	-
Shanstar - Cran 2 Supernatant only	-	0	0	0	0	0	24	89	TNTC	-
Shanstar - Cran 2 Suspended Product	-	0	0	0	0	*25*	+	TNTC	TNTC	-

A significant amount of articles published recently has associated the cranberry's antimicrobial properties with the "anti-adherence" capability as a primary mode of action to aid in the alleviation of urinary tract infections (Sobota, 1984; Ofek *et al.,* 1996; and Zafriri *et al.,* 1989). Through the assay employed for determining the antimicrobial activity of the Shanstar product, table 2 contains data that supplies evidence to support the anti-adherence activity of the compounds found in cranberries. After the Shanstar product is incubated with *E. coli* cells, two methods can be performed to express the number of viable cell remaining. In the first method, the Shanstar is mixed into suspension and applied directly to an agar plate (suspended product). In the second method, the Shanstar is separated from the saline by gravity and only the saline portion is applied to the plate (supernatant only). The results relating to the suspended product show a lower titer when compared to the supernatant only. This suggests that the polyphenolic compounds in the Shanstar-cranberry are adhering to the *E. coli* cells and acting as an anchor to pull the cells out of solution. This suggests that both anti-adherence and bactericidal properties are present. The bactericidal action may take place as a secondary function to the anti-adhesion activity.

Additionally, saline is utilized to allow the Shanstar product to establish a pH which is representative of the contained compounds. Preliminary analysis indicates that the antimicrobial **effectiveness is reduced** upon increasing the pH of the product to a pH **near 7.0.**

Table 3. **Shanstar - Cranberry Titer Relative to the Concentration of Bacterial Cells**

Samples	1:16	1:32	1:64	1:128	1:256	1:512
$1x10^3$cells	0	0	0	9	5	+100
$1x10^4$cells	0	0	0	8	+97	++
$1x10^5$cells	0	0	0	87	++	+++

+ = slight excessive growth, ++ = growth like 10^3 control, +++ = growth like 10^4 control

The effectiveness of the Shanstar-cranberry with relation to the number of cells is shown in table 3. These results represent the supernatant only method and the test is based against $1x10^5$ cells, $1x10^4$ cells, and $1x10^3$ cells. The results suggest that the Shanstar-cranberry product has the ability to inhibit **10 times** more *E. coli* cells by only **doubling** the amount of product.

Shanstar-cranberry's effectiveness has also been tested against a variety of different bacteria. The data in table 4. indicates that the product has microbial inhibitory activity against various bacteria other than *E. coli.* There are differing results among the bacteria analyzed, but all were susceptible to the Shanstar-cranberry when inoculated at a cell concentration of approximately $1x10^3$cells/mL.

Table 4. **Titer range of Various Bacteria Post-Shanstar Treatment (1)**
Data represents cell concentrations at ~$1x10^3$cells/mL

Bacteria	ATCC strain number	Min. titer	Max. titer
Escherichia coli	**25922**	**64**	**256**
Salmonella cholerasuis	14028	32	128
Staphylococcus epidermidis	12228	32	128
Serratia marcescens	8100	64	256
Pseudomonas aeruginosa	27853	128	512
Bacillus subtilis	6633	512	1024
Pseudomonas fluorescens	13525	32	128
Yersinia enterocolitica	23715	512	1024
Enterococcus faecalis	51299	16	64

*Titer range represents variability in inoculum concentration due to dilution (cell concentration range: $5x10^2$ cells/mL to $1.5x10^3$ cells/mL)

(1) – Titer Range is Affected by the pH.

References:

Ofek, I, J. Goldhar, and N. Sharon.1996. Anti-*Escherichia coli* adhesin activity of Cranberry and Blueberry Juices. Toward Anti-Adhesion Therapy for Microbial Diseases. Plenum Press, New York.p179-183.

Zafriri, D., I. Ofek, R. Adar, M. Pocino, and N. Sharon. 1989. Inhibitory activity of cranberry juice on adherence of type 1 and type P fimbriated *Escherichia coli* to eukaryotic cells. Ant. Microbial. Agt. Chem. 33:92-98

Sobota, A. E. 1984. Inhibition of bacterial adherence by cranberry juice: Potential use for treatment of urinary tract infections. J. Urol. 131:1013-1016.

5.1.B Method: Antimicrobial Susceptibility Protocol (MIC/MBC)

OBJECTIVE:

To determine the antimicrobial potency of the sample against *Escherichia coli*. The antimicrobial assay has been developed to assess the extent at which the product can be diluted and still maintain its inhibitory activity.

PROCEDURE:

The antimicrobial assay is a derivation of the Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC) Assays typically utilized to determine antimicrobial potency.

1. Inoculation of culture:

The assay is initiated by inoculating a starter culture. With a sterile inoculating loop, inoculate a 5.0mL of tryptic soy broth culture with *E. coli* 25922 in a 15 x100 sterile test tube and incubate for 2 - 3 hours at 37°C, or overnight at 30°C, with shaking at 200 rpm (orbital shaker, VWR - 57018-75). The culture should be grown to mid-log phase and not to exceed late log-phase. The chosen strain of *E. coli* is non-pathogenic and recommended by the Association of Official Analytical Chemists for use in most quality control assays.

2. Sample Preparation:

The product samples are prepared by setting up ten, two-fold dilutions, each in a 15mL conical tube (Fisher Scientific). The most concentrated sample is a 1:2 dilution (50% w/v) of the product suspended in sterile 0.85% NaCl (saline), ending with a 1:1024 dilution.

3. Cell Preparation:

The concentration of cells used to evaluate the effectiveness of the product can range from $1x10^2$cells/mL to $1x10^5$cells/mL, depending on the sample to be tested. The level of cell concentration utilized is relative to the effectiveness of the competitor's product. This concentration is determined by attaining a readable set of dilutions in which a titer can be assessed. The culture is analyzed spectrophotometrically at OD_{550} and an absorbance reading at 0.5 is approximately $1x10^8$cells/mL (Miller, 1972). The culture is diluted with sterile saline to obtain a stock concentration of bacteria.

4. Inoculation of Sample:

100µL of the stock is added to each dilution tube. The final cell concentration per milliliter will be approximately 10 times less than that of the stock. The tubes are incubated at room temperature for 30 minutes and the sample is kept in suspension by shaking (> 350 rpm with an orbital shaker, VWR Scientific).

5. Plating : Method I - Applying the Supernatant Only

After the 30 minute incubation, the tubes are incubated for 5 minutes at room temperature without shaking, this is to allow settling of the insoluble product and only done when using plating method I. 1.0mL of supernatant is transferred to a tryptic soy agar plate and spread with a sterile spreading utensil. The plates are allowed to set for about 5 minutes to enable the saline to absorb into the agar. The plates are inverted and incubated overnight at 37°C.

6. Plating: Method II - Applying the Suspended Product

With this technique, the dilution samples are not incubated for an additional 5 minutes, the contents of the tube are mixed immediately after the 30 minute incubation by pipetting up and down to suspend the product. 1.0mL from each dilution is transferred to a tryptic soy agar plate. The plates require between 5 to 10 minutes to allow the sample

to absorb into the agar. This must be done on a level surface. The plates are inverted and incubated overnight at 37°C.

7. Controls:

The titer achieved by the product is directly related to the concentration of cells used in the assay. To ensure the proper targeted concentration was applied, a ten-fold serial dilution must be prepared from the stock culture used to inoculate the dilution samples. The serial dilution must go as low as $1x10^1$cells/mL. Attaining approximately 10 colonies on the $1x10^1$cells/mL plate would verify that the stock culture accurately reflects its targeted concentration. 1.0mL of each dilution is applied to a tryptic soy agar plate and incubated overnight in the same manner as the experimental plates. A second control is prepared by plating an uninoculated sample at the 1:16 or 1:32 dilution.

8. Result:

The dilution sample in which there is less than 0.5% of the original inoculum is expressed as the titer.

BIBLIOGRAPHY:

Miller, J. H., Experiments in Molecular Genetics. Cold Spring Harbor Laboratory. 1972. P.31.

5.2 In vitro antimicrobial testing: MIC and MLC at pH 5.0

5.2.A Minimum Inhibitory Concentration Test Report

OBJECTIVE:
This test was performed to determine the antimicrobial activity of Shanstar Cranberry Extract against *Escherichia coli.* The test protocol establishes and maintains standard conditions for all controls and samples challenged. The inhibitory activity is quantified by enumerating the remaining viable cells (expressed as colony forming units) through the spread plate method.

PROTOCOL: See the attached MIC protocol.

STRAIN: *Escherichia coli* 25922. This strain is non-pathogenic and recommended by: the National Committee for Clinical Laboratory Standards (NCCLS) in Antimicrobial Susceptibility and Bactericidal tests, the Association of Official Analytical Chemists (A.O.A.C) for use in most quality control assays, and used as a quality control strain by many leading microbiological media companies.

RESULTS:
The tube dilution method of determining the minimum inhibitory concentration of an antimicrobial is widely employed as a means of analyzing antibiotics in the medical technology and microbiology fields. For the analysis of ShanStar Cranberry Extract, standard testing conditions were established to determine the minimum inhibitory concentration. The preliminary studies assess ShanStar's antimicrobial activity against *E. coli* at an initial inoculum of ~$1x10^5$ cells/mL. All dilution samples of the extract were prepared in a Trypticase Soy Phosphate Buffer Broth (T.S.B.) and adjusted to pH 5.0 with 1N HCl. The contact time, in which ShanStar was exposed to the *E. coli* cells, was 18 hours at 30°C with shaking at ~180rpm. The inhibitory activity of ShanStar was [redacted]

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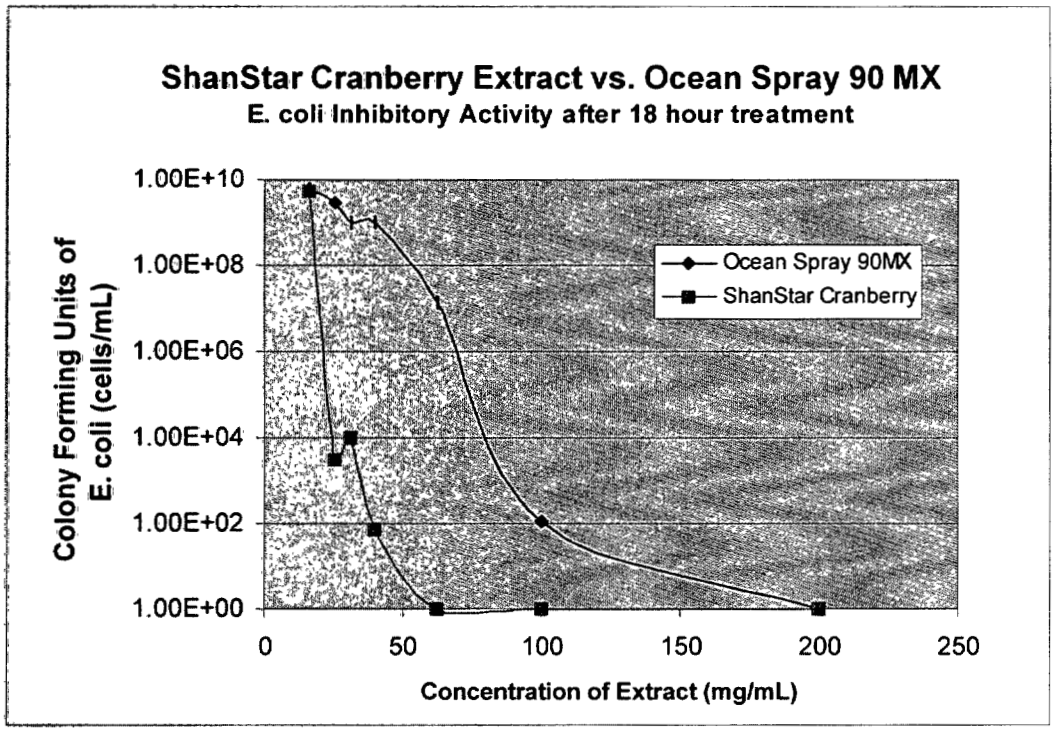
quantified by the spread plate method to enumerate the viable *E. coli* cells after the 18 hour exposure period. The positive controls used to substantiate the data were cells grown only in TSB at pH 5.0 and pH 7.2. PVPP only was also tested at a concentration of 31.2mg/mL to verify any contributory affect supplied by the carrier. The results obtained are displayed in Table 1 and expressed graphically in Graph 1 to establish a tenative inhibition curve.

Table 1: ShanStar Cranberry vs. Ocean Spray inhibitory activity at various concentrations

Sample concentration (mg/mL broth)	15.6	25.0	31.2	40.0	62.4	100	200
Ocean Spray 90MX	6.00E+09	2.80E+09	1.00E+09	1.00E+09	1.34E+07	112.5	0
ShanStar Cranberry	5.00E+09	2.95E+03	9.67E+03	72	0	0	0

Graph 1: Inhibitory effect of ShanStar vs. Ocean Spray 90MX at pH 5.0



The minimum inhibitory concentration (MIC) is defined as the concentration which inhibits any growth of the initial $1x10^5$ cells/mL, resulting in $\sim 1x10^5$ cells/mL viable cells after the 18 hour incubation. From the graph, the minimum inhibitory concentration of ShanStar Cranberry appears to be between 20 to 30mg/mL, while the Ocean Spray sample is approximately 80mg/mL. Further studies are required to confidently establish the MIC, this is due to the wide standard deviation values attained from preliminary statistical analysis.

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The data for the positive controls were very similar and are expressed in table 2.

Table 2.

Control Samples	cells/milliliter	Std. Dev.
Cells only at pH 5.0	4.9×10^9	$\pm 1.8 \times 10^9$
Cells only at pH 7.2	9.8×10^9	$\pm 2.3 \times 10^9$
PVPP only at pH 5.0 (31.2mg/mL)	3.1×10^9	$\pm 0.97 \times 10^8$

From this data, there is a 3 to 4 times greater level of inhibition, of 10^5 *E. coli* cells, in ShanStar than in Ocean Spray 90MX. This is derived from comparing the amount of extract required to inhibit 10^5 *E. coli* cells. These results support the other data relating to ShanStar Cranberry and correspond to the 5 to 6 times greater phenolic content when compared to Ocean Spray 90 MX.

5.2.B MIC/MLC Protocol

Pretest Preparation:

a. Inoculate 5.0mL Trypticase Soy (T. S.) broth with *Escherichia coli* (ATCC 25922) for overnight incubation at 30°C with shaking.
b. Modified T. S. broth at pH 5.0 – to prepare 100mL, mix components and autoclave:
   - 50.95mL 0.1M Citric acid
   - 49.01mL 0.2M Na_2HPO_4
   - 3.0g T. S. broth base
c. Modified T. S. broth at pH 6.0 – to prepare 100mL, mix components and autoclave:
   - 38.77mL 0.1M Citric acid
   - 61.23mL 0.2M Na_2HPO_4
   - 3.0g T. S. broth base
d. Modified T. S. broth at pH 7.2 – to prepare 100mL, mix components and autoclave:
   - 14.24mL 0.1M Citric acid
   - 85.73mL 0.2M Na_2HPO_4
   - 3.0g T. S. broth base

e. Unmodified T. S. broth at pH 7.2 – to prepare 100mL, mix and autoclave:

- 100mL distilled water
- 3.0g T. S. broth base

Sample Preparation and Experiment:

a. Two-fold serial dilutions are prepared from an antimicrobial sample in modified T. S. broth to a volume of 5.0mL. Initial sample is 50% w/v (2.5g added to 2.5mL broth).

b. Analyze the pH of each dilution, all dilutions must have the same pH level as the broth in which it was prepared.

c. Inoculate each dilution with 50uL from a $1x10^7$ cells/mL stock to obtain an inoculum concentration of $1x10^5$ cells/ml.

d. Incubate the samples for 18 hours with shaking at 160 rpm and 30°C.

e. Cell growth controls: inoculate all appropriate broths with same amount of *E. coli,* in the absense of any antimicrobial samples.

f. After the 18 hour incubation, perform a ten-fold serial dilution on each antimicrobial dilution from each antimicrobial sample tested; a dilution range from 10^{-1} to 10^{-9}.

g. Overlay T. S. agar plates with 500uL from each ten-fold dilution to determine the level of inhibitory activity on the initial inoculum, due to the presence of the tested concentration of antimicrobial agent. This step is performed in triplicate for each ten-fold dilution.

h. Invert the plates and incubate at 37°C.

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5.3 HPLC Total Phenolic Testing

5.3.A Introduction

Testing of the active compounds in herbal supplements has been a growing concern in the nutraceutical industry. Most commercial brands often do not contain the adequate level of the active ingredient as reported by the Los Angeles Times. In the cranberry case, the effect of preventing the urinary tract infection was initially attributed to fruit's acidic nature. Because of that, the method used in the juice industry to identify and detect adulteration in cranberry juice cocktail has been also used in assessing the authenticity and potency of cranberry extract. This evaluation is based on the organic acid compositions and their ratios, especially quinic acid, which is a unique acid in cranberries and an expensive ingredient. Recently, the New England Journal of Medicine (Vol. 339, Oct. 1998) has reported that proanthocyanidins are in fact the bio-active compounds responsible for preventing *E. coli* from adhering to the urinary tract.

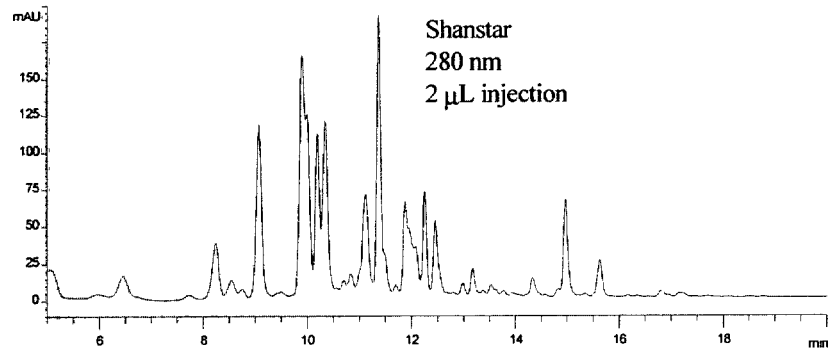
Since organic acids are not the active compounds, technologies that minimize the content of organic acids and oligosaccharides (sucrose, glucose, fructose) are needed to produce an extract that is more concentrated in active compounds. One of the major advantages of the Shanstar technology is to reduce the amount of acid and oligosaccharides in the product. Therefore, the potency and the standardization of Shanstar should not be measured based on organic acids. It is worth mentioning that the oligosaccharides and organic acids account for over 90% of the total soluble solids present in fruit juices. In other words, over 90% of the contents of most commercial pills available in the market are composed of non-active ingredients.

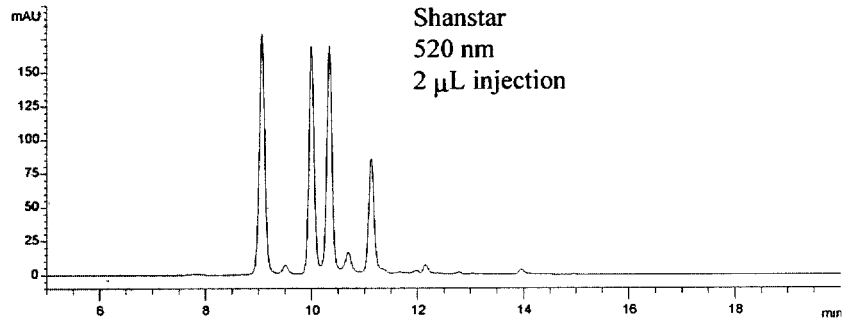
The objective of this report is to present a different method for checking the source of the active ingredients and for measuring the relative amount of active compounds of Shanstar when compared with the cranberry juice cocktail. The HPLC polyphenolic profile is a convenient method because the chromatogram of anthocyanins not only indicate whether the anthocyanin pattern is typical of cranberry, but also the total peak area in the chromatogram at the UV spectra (280 nm) provides a relative measurement of active compounds. The total peak area is constituted by all phenolic compounds, including the proanthocyanidins.

5.3.B Objective

To determine the polyphenolic profiles of Shanstar by HPLC and to compare the relative amount of phenolic compounds of Shanstar with those of cranberry juice cocktail and two other prominent cranberry products.

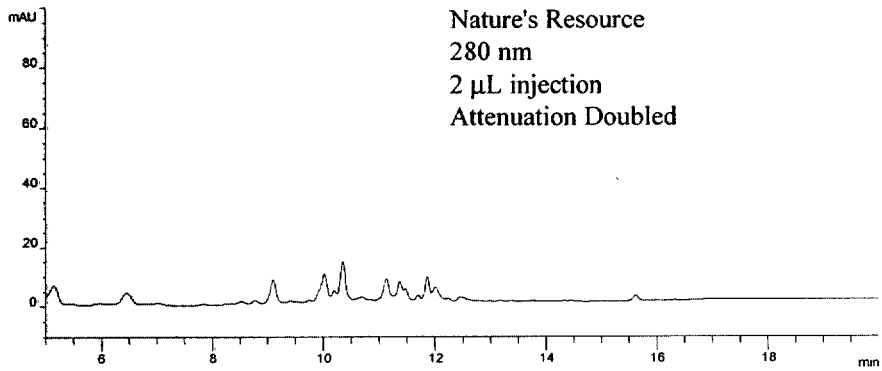
5.3.C Results





Nature's Resources

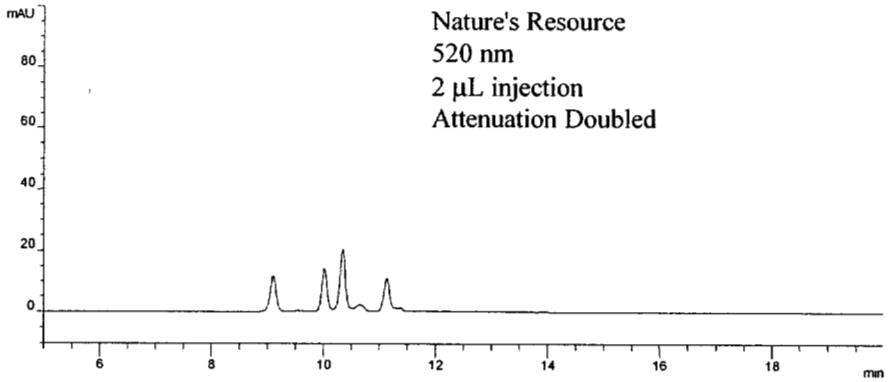
- Because of the relatively low concentration of compounds in comparison to Shanstar, the sensitivity of the detector was doubled.



000536

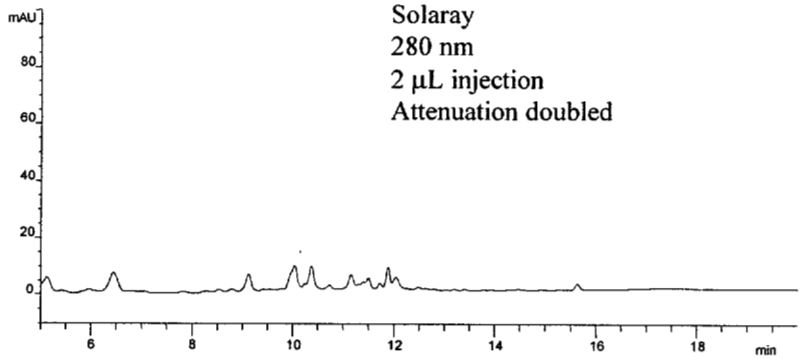
[redacted]

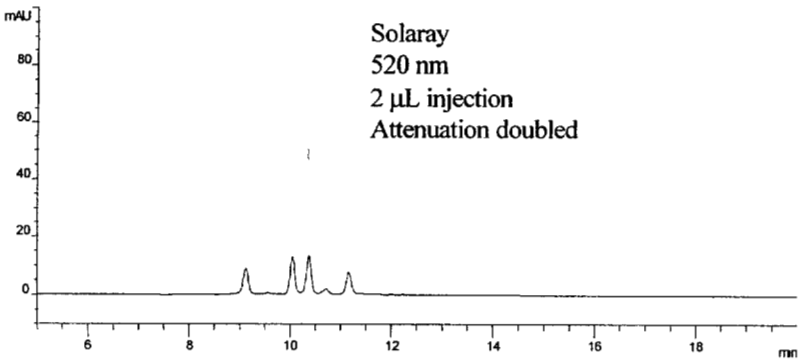
[redacted]



Solaray

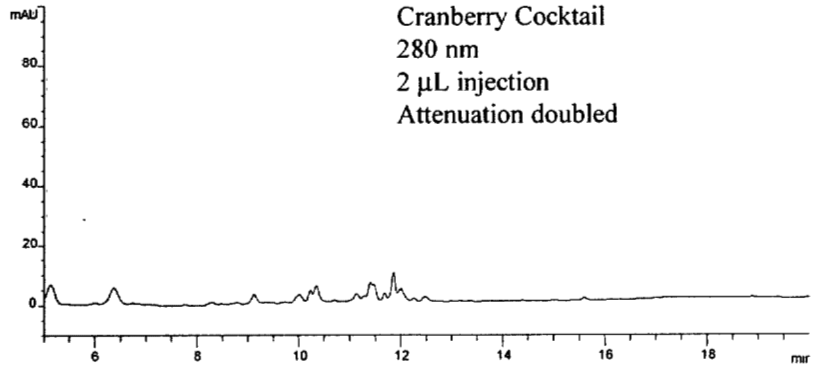
- Because of the relatively low concentration of compounds in comparison to the Shanstar product, the sensitivity of the detection was doubled.

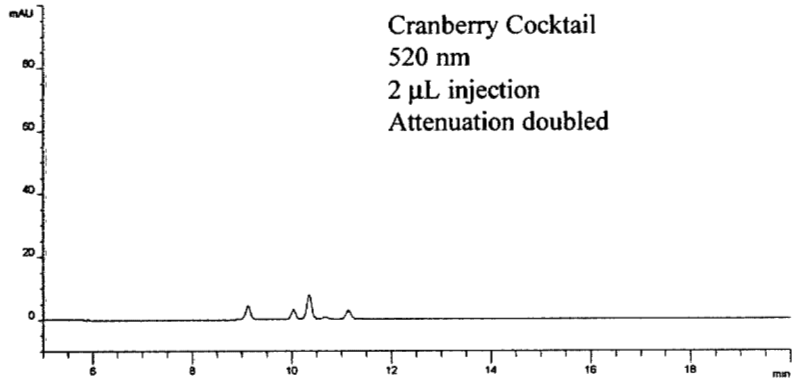




Cranberry Cocktail

- Because of the relatively low concentration of compounds in comparison to Shanstar , the sensitivity of detection was doubled.





5.3.D Discussion

The typical cranberry pattern and the presence of the anthocyanins: cyanidin-3-galactoside (cyd-3-gal), cyanidin-3-glucoside (cyd-3-glu), cyanidin-3-arabinoside (cyd-3-ara), peonidin-3-galactoside (pnd-3-gal), peonidin-3-glucoside (pnd-3-glu), and peonidin-3-arabinoside (pnd-3-ara), are shown in the visible spectra (520 nm) for Shanstar. The same patterns are found in all three competitors' samples.

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The proanthocyanidins, the active compounds reported in the New England Journal of Medicine, are colorless and shown in the UV spectra (280 nm). The relative amount of phenolic compounds, including proanthocyanidins was evaluated by integrating the total area of each chromatogram at 280 nm from 5 to 20 minutes as shown in Table 1 and Figure 1 [redacted]

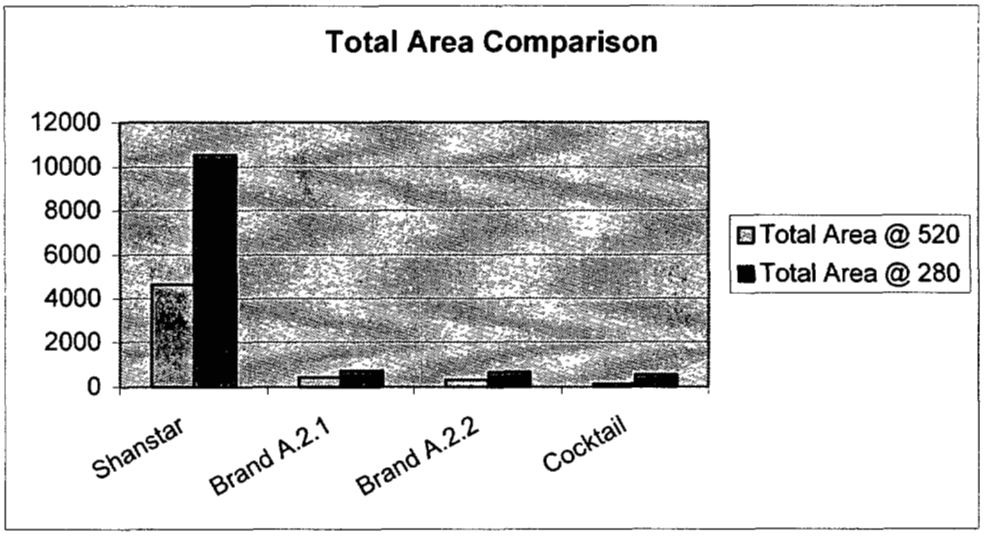
Table 1 - Total Area Comparison

280 nm		520 nm	
Sample	**Total Area**	**Sample**	**Total Area**
Shanstar	10561.7	Shanstar	4662.7
Nature's Resource	736.4	Nature's Resource	427.5
SOLARAY	675.4	SOLARAY	318.0
Cranberry Cocktail	545.6	Cranberry Cocktail	137.3

Figure 1

Note: Integrated from five to 20 minutes.

As shown in Table 1, the equivalent sample sizes for cranberry cocktail and Brand 3 required to receive approximately the same amount of proanthocyanidins in gram of Shanstar are 365.0 ml and 10.0 grams, respectively. In the graph below A.2.1 refers to Nature's Resource and A.2.2 refers to Solaray.



000539

5.4 Animal Studies

5.4.A Summary of Oral toxicity of ShanStar Cranberry extract

ShanStar™ Cranberry Extract is a mixture of cranberry polyphenols adsorbed on pharmaceutical grade polyvinylpolypyrrolidone (PVPP). The Joint Food and Agricultural Organization/World Health Organization Expert Committee on Food Additives established an Allowable Daily Intake for PVPP limited only by Good Manufacturing Practice (GMP)[1].

Cranberry polyphenols have a long history of consumption in the form of whole cranberries, cranberry sauces, and cranberry juice cocktails. The extraction process (patent pending) used by ShanStar Biotech, Incorporated has no effect on the chemistry of cranberry polyphenols. In addition, organic solvents are not used in the process.
In order to confirm that the toxicology of the cranberry polyphenols are unchanged during the extraction process, a short term acute toxicity study was conducted using rats and ShanStar Cranberry extract. An LD_{50} of >5000 mg/kg body weight for this material. A Quality Assured report of this study conducted at Product Safety Labs of East Brunswick, New Jersey, is available from ShanStar Biotech, Incorporated.

Dr. Gareth Templeman
Vice President, Corporate Services
Vice President Regulatory Affairs and Quality
Cliffstar Corporation*

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* Cliffstar wholly owns ShanStar Biotech, Incorporated.

[1] 27th report of the JECFA, Geneva, WHO Technical Report, Series Number 696, 1983.

5.4.B Acute Oral Toxicity of ShanStar Cranberry extract

See copy of original in following pages.

000541

psl **product safety labs**
725 Cranbury Road • East Brunswick, New Jersey 08816-3206 • 732-254-9200 • 800-425-0002 • Fax-732-254-6736

5.4.B Acute Oral Toxicity of Shan Star Cranberry extract

March 26, 1999

Mr. Yang Wei
L&S CRANBERRY MANAGEMENT
One Cliffstar Avenue
Dunkirk, NY 14048

Dear Mr. Wei:

Enclosed please find the following FINAL REPORT for Cranberry Extract/Shanstar:

Study # 7190 - P320 Acute Oral Toxicity Study

Invoice will be sent under separate cover.

Thank you for your confidence in Product Safety Labs. Please contact George Moore, our Study Director, for comments and/or questions.

Sincerely,

PRODUCT SAFETY LABS

Carol Ann Soden
Administrative Assistant

Enc.

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psl product safety labs
725 Cranbury Road • East Brunswick, New Jersey 08816-3206 • 732-254-9200 • 800-425-0002 • Fax-732-254-6736

ACUTE ORAL TOXICITY STUDY IN RATS - LIMIT TEST

PROTOCOL NO.:	P320
AGENCY:	FDA
STUDY NUMBER:	7190
SPONSOR:	L&S CRANBERRY MANAGEMENT INC. One Cliffstar Avenue Dunkirk, NY 14048
TEST ARTICLE IDENTIFICATION:	Cranberry Extract/Shanstar Lot #TG 1298
TEST ARTICLE DESCRIPTION:	Cranberry red powder
DATE RECEIVED:	February 8, 1999
PSL REFERENCE NO.:	E90208-1R
DATE OF PROTOCOL APPROVAL:	February 12, 1999
DATES OF TEST:	February 16 - March 2, 1999
STUDY COMPLETION DATE:	March 26, 1999
NOTEBOOK NO.:	99-14; pages 353-369

1. PURPOSE

To provide information on health hazards likely to arise from a short-term exposure to Cranberry Extract/Shanstar by the oral route.

2. SUMMARY

An acute oral toxicity test was conducted with rats to determine the potential for Cranberry Extract/Shanstar to produce toxicity from a single dose via the oral route. Based on the results of this study, the single dose acute oral LD_{50} of the test article is greater than 5,000 mg/kg of bodyweight when administered as a 30% w/w suspension in distilled water.

Five thousand milligrams of the test article per kilogram of bodyweight was administered to ten healthy rats by oral gavage. The animals were observed for mortality, signs of gross toxicity, and behavioral changes at least once daily for 14 days. Bodyweights were recorded prior to administration and again on Days 7 and 14 (termination). Necropsies were performed on all animals at terminal sacrifice.

000543

All animals survived, gained weight and appeared active and healthy. There were no signs of gross toxicity, adverse pharmacologic effects or abnormal behavior. Gross necropsy findings at terminal sacrifice were unremarkable.

3. MATERIALS

A. Test Article

The test article identified as Cranberry Extract/Shanstar, Lot #TG 1298 was received on February 8, 1999 and was further identified with PSL Reference Number E90208-1R. The test article was a cranberry red powder and was stored at room temperature. The sample was administered as a 30% w/w suspension in distilled water. Preliminary solubility testing conducted by PSL indicated that suspensions in excess of 30% (i.e. 40 or 50%) were too viscous to be administered properly. Documentation of the methods of synthesis, fabrication, or derivation of the test article is retained by the sponsor.

Characterization of the test article provided to Product Safety Labs by the sponsor was:

Composition: Active compounds: phenols – 50%
Other ingredients: USP polymer – 50%

pH: 2.6-3.0 (at 5% dilution)

Solubility: Active compound is soluble in methanol and ethanol; polymer is insoluble

Stability: Test article is expected to be stable for the duration of testing

Expiration Date: December 30, 1999

B. Animals

3.B.1 Number of Animals: 10

3.B.2 Sex: 5 males and 5 females

3.B.3 Species/Strain: Rat/Sprague-Dawley derived, albino

3.B.4 Age/Bodyweight: Young adult/males 182-203 grams and females 150-167 grams at initiation

3.B.5 Source: Received from Ace Animals, Inc., Boyertown, PA on February 9, 1999

4. METHODS

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A. Husbandry

4.A.1 Housing: The animals were singly housed in suspended stainless steel caging with mesh floors which conform to the size recommendations in the most recent *Guide for the Care and Use of Laboratory Animals DHEW (NIH)*. Litter paper was placed beneath the cage and was changed at least three times per week.

4.A.2 Animal Room: Temperature Range: 17-23°C

4.A.3 Photoperiod: 12 hour light/dark cycle

4.A.4 Acclimation Period: 7 days

4.A.5 Food: Purina Rodent Chow #5012

4.A.6 Water: Filtered tap water was supplied *ad libitum* by an automatic water dispensing system.

4.A.7 Contaminants: There were no known contaminants reasonably expected to be found in the food or water at levels which would have interfered with the results of this study. Analyses of the food and water are conducted at least once a year and the records are kept on file at Product Safety Labs. The dates of the most recent analyses are presented in Appendix A.

B. Identification:

4.B.1 Cage: Each cage was identified with a cage card indicating at least the study number and identification and sex of the animals.

4.B.2 Animal: A number was allocated to each rat on receipt and a stainless steel ear tag bearing this number was attached to the rat. This number, together with a sequential animal number assigned to study 7190, constituted unique identification.

5. PROCEDURE

A. Selection of Animals

Prior to dosing, a group of animals was fasted for approximately 21.5 hours by removing feed from their cages. During the fasting period, the rats were examined for health and weighed (initial). Ten (five male and five female) healthy rats were selected for test.

B. Dose Calculations

Individual doses were calculated based on the initial bodyweights, taking into account the specific gravity (determined by PSL) and concentration of the test suspension.

C. Dosing

Each animal received 5,000 mg/kg of the test article by intubation using a stainless steel ball-tipped gavage needle attached to an appropriate syringe. The test article was administered as a 30% w/w suspension in distilled water. After administration, each animal was returned to its designated cage. Feed was replaced approximately 3.5 hours after dosing. The day of administration was considered Day zero of the study.

D. Bodyweights

Individual bodyweights of the animals were recorded prior to test article administration (initial) and again on Days 7 and 14 (termination) (See Table 1).

000545

E. Cage-Side Observations

The animals were observed for mortality, signs of gross toxicity, and behavioral changes at 1 and 4 hours post-dosing and at least once daily thereafter for 14 days. Observations included gross

evaluation of skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behavior pattern. Particular attention was directed to observation of tremors, convulsions, salivation, diarrhea and coma (See Table 2).

F. Necropsy

All rats were euthanized via CO_2 inhalation on Day 14. Gross necropsies were performed on all animals. Tissues and organs of the thoracic and abdominal cavities were examined (See Table 3).

6. STUDY CONDUCT

This study was conducted at Product Safety Labs, 725 Cranbury Road, East Brunswick, NJ 08816, to comply with the good laboratory practices as defined in 21 CFR 58: U.S. FDA Good Laboratory Practice Standards and in accordance with Toxicological Principles for the Safety Assessment of Direct Food Additives and Color Additives Used in Food, Guidelines for Acute Oral LD_{50} Toxicity Studies.

7. QUALITY ASSURANCE

The final report was audited for agreement with the raw data records and for compliance with the protocol, Product Safety Labs Standard Operating Procedures and appropriate Good Laboratory Practice Standards. Dates of inspections and audits performed during the study, and the dates of reporting of the inspection and audit findings to the Study Director and Facility Management are presented in the Quality Assurance Statement.

8. DEVIATIONS FROM FINAL PROTOCOL

None

9. RECORDS TO BE MAINTAINED

A copy of this signed report, together with the protocol and all raw data generated at Product Safety Labs, is retained in the Product Safety Labs Archives.

10. RESULTS

All animals survived, gained weight and appeared active and healthy. There were no signs of gross toxicity, adverse pharmacologic effects or abnormal behavior. No gross abnormalities were noted for the animals when necropsied at the conclusion of the 14-day observation period.

11. CONCLUSION

The single dose acute oral LD_{50} of Cranberry Extract/Shanstar is greater than 5,000 mg/kg of bodyweight when administered as a 30% w/w suspension in distilled water.

SIGNATURES

Cranberry Extract/Shanstar

This study meets the requirements of the Good Laboratory Practice Standards as defined by 21 CFR 58: U.S. FDA with the following exception: The stability, characterization, identity and verification of the test article concentration as received and tested are the responsibility of the study sponsor.

March 26 19

George E. Moore, B.S.
Study Director

Date

We the undersigned declare that the methods, results and data contained in this report faithfully reflect the procedures used and raw data collected during the study.

Mar 26, 1999

Gary Wnorowski, B.A.
Laboratory Manager

Date

March 26 1999

Jacek Odhalski, D.V.M.
Principal Toxicology Technician

Date

000547

[redacted]

TABLE 1: INDIVIDUAL BODYWEIGHT AND DOSAGE

Animal No.	Sex	Bodyweight (g)			Dose[1]
		Initial	Day 7	Day 14	ml
3291	M	200	300	346	3.1
3292	M	203	287	337	3.1
3293	M	197	273	331	3.0
3294	M	201	278	310	3.1
3295	M	182	248	287	2.8
3296	F	150	190	226	2.3
3297	F	164	196	222	2.5
3298	F	167	200	225	2.5
3299	F	159	202	230	2.4
3300	F	167	210	231	2.5

[1] Administered as a 30% w/w suspension in distilled water. Specific Gravity - 1.092 g/ml.

[redacted]

TABLE 2: INDIVIDUAL CAGE-SIDE OBSERVATIONS

Animal Number	Finding	Day of Occurrence
MALES		
3291-3295	Active and healthy	0-14
FEMALES		
3296-3300	Active and healthy	0-14

[redacted]

TABLE 3: INDIVIDUAL NECROPSY OBSERVATIONS

Animal Number	Tissue	Findings
MALES		
3291-3295	All tissues/organs	No gross abnormalities
FEMALES		
3296-3300	All tissues/organs	No gross abnormalities

[redacted]

APPENDIX A: FEED AND WATER ANALYSES

Animal feed analysis independently performed on March 17, 1998 for the presence of the following contaminants:

Aldrin	Ethyl Parathion
BHC	Heptachlor
Chlordane	Heptachlor Epoxide
DDD	Hexachlorobenzene - HCB
DDE	Lindane
DDT	Malathion
Diazinon	Methoxychlor
Dieldrin	Methyl Parathion
Endosulfan I & II	Mirex
Endosulfan Sulfate	Parathion
Endrin	PCB
Endrin aldehyde	Toxaphene
Ethion	

LABORATORY: WOODSON-TENENT LABORATORIES
345 Adams Avenue
P.O. Box 2135
Memphis, TN 38101

Water analysis performed as of January 19, 1998 for NJDEPE Safe Drinking Water Act parameters.

LABORATORIES: NEW JERSEY LABORATORIES
NJDEPE LAB I.D. #15001
A.A. Labs Division
222 Easton Avenue
New Brunswick, NJ 08901

SILLIKER LABORATORIES
OF NEW JERSEY, INC.
400 South Avenue
Garwood, NJ 07027

Results of feed and water analysis for possible contaminants: Acceptable; none detected or within regulatory standards.

[redacted]

[redacted]

QUALITY ASSURANCE INSPECTIONS STATEMENT

The Quality Assurance Unit randomly selects intervals for QA inspections prior to study initiation. Records of the findings of these inspections are kept on file. The summary below provides verification of statements made in the final report section that addresses Quality Assurance audits.

Inspections were made of:

DATE	PROCEDURE INSPECTED
2/16/99	Test substance preparation
3/2/99	Terminal bodyweights
3/26/99	Raw data
3/26/99	Draft report
3/26/99	Final report

Findings reported to: Study Director 3/22, 3/26/99

Management 3/22, 3/26/99

Frank Fielder, B.S.
Quality Assurance Supervisor

[redacted]

[redacted]

5.4.C Summary of studies on Allowable Daily Intake (ADI) of PVPP cited by JECFA/FAO/WHO

"A Submission by International Specialty Products (Wayne, NJ, USA) to the Joint Expert Committee on Food Additives of the FAO/WHO on the safety of PVPP and to establish an Allowable Daily Intake"

Various studies conducted by ISP are summarized below. More complete information is available from International Specialty Products, Wayne, NJ, USA.

ACUTE ORAL TOXICITY

Rats and mice were fed 5g/kg (bodyweight) POLYVINYLPOLYPYRROLIDONE.No abnormal symptoms or deaths were observed after 24, 48 or 72 hours.

28-DAY FEEDING STUDY IN RATS:

Five groups of 40 rats each (equally divided by sex) were dosed for 4 weeks at 0, 1, 2.5, 5 or 10% PVPP in their feed followed by 2 weeks of no PVPP in the diet.At the 10% level, a slight bodyweight decrease was observed.Dose-dependent urinary casts in the urine sediment of females was reversible when PVPP feeding stopped.Clinical chemistry and hematology, necropsy results and observations showed no differences from control animals.PVPP was not deposited in the small intestine mucosa or mesenteric lymph nodes.

90-DAY FEEDING STUDY IN RATS:

Three groups of 40 Wistar strain rats (equally divided by sex) were fed diets for 90 days at 0, 2, or 10% PVPP.No compound related effects were noted in behavior, appearance, food consumption, feed efficacy, bodyweight gain, clinical studies (hematology, clinical biochemistry and urinalysis), necropsy or histopathology results.

28-DAY FEEDING STUDY IN DOGS:

Three groups of 6 beagle dogs were dosed by stomach tube with an aqueous suspension of PVPP at 1000, 2000 or 5050 mg/kg bodyweight for 4 weeks.Control animals received water or a cellulose suspension (400 mg/kg bodyweight).No compound related effects were noted in food and water consumption, hematology, clinical chemistry, urinalysis and histopathology.No accumulation of PVPP was found in the liver, kidneys or mesenteric lymph nodes.

180-DAY FEEDING STUDY IN DOGS:

Three groups of 8 beagle dogs were dosed by stomach tube with an aqueous suspension of 300, 1200 or 4888 mg/kg bodyweight for 26 weeks.Control animals received water or a cellulose suspension (4800 mg/kg bodyweight).Because the high dose level dogs showed increasing emesis during weeks 7 - 12, divided doses were given.No compound related effects were noted in behavior, food and water consumption, growth, hematology, clinical chemistry or urinalysis results, electrocardiography, opthamology, auditory tests, gross or histopathology.No PVPP deposition or storage was found in the liver, kidneys or mesenteric lymph nodes.

TERATOGENICITY

Three groups of 26 pregnant SPF rats were dosed by gavage with suspensions of PVPP (0, 1000 or 3000 mg/kg bodyweight) in carboxymethyl cellulose from day 6 to 15 and sacrificed on day 20 of gestation.One group was left untreated.A slight reversible decrease in weight gain occurred in the high-dose group and the weights of the fetuses of all test animals were slightly lower than of controls.No changes in fetal length were observed.There were no significant clinical symptoms, maternal deaths and no compound-related necropsy findings for the dams.Conception rate and live and dead implantations were not affected.The fetuses displayed no compound-related skeletal or visceral abnormalities.

Two additional groups of 12 pregnant SPF rats received the same dose levels (1000 or 3000 mg/kg) from day 15 to day 21 post-parturition.Weight gain, mortality, delivery time and litter size of the test animals were comparable to the control.No macroscopic compound-related changes were observed in the mothers.Pups of treated animals showed similar mortality, bodyweight and behavior as the control.Autopsy revealed no compound-related skeletal or visceral abnormalities.Effects noted were lower heart weight of male and female pups and lower spleen weight of male pups in the 3000 ppm (0.3%) group, as well as some changes in the males' kidneys and females' livers.No other compound-related effects were noted in the dams or pups.

PHARMACOKINETICS

Studies on ^{14}C-labeled PVPP in the rat showed almost no absorption or gastrointestinal accumulation of orally dosed PVPP and no biliary excretion.

Six Sprague-Dawley rats received an oral dose of 250 mg
^{14}C-PVPP by gastric intubation.The PVPP used had been treated to remove polymers of less than 10,000 m.w.Approximately 0.128% of the ^{14}C-PVPP was excreted in the urine, most being excreted within the first 24 hours.80-99% of the ^{14}C was recovered in the feces in the 12-24 hour period.Less than 0.1% of the ^{14}C was recovered in the carcass, most being in the G.I. tract.There was no evidence of preferential binding of the ^{14}C to any organ or tissue.

Long-term clinical studies show that PVPP is unabsorbed in the gastrointestinal tract.No inherent pharmacological action, therefore, would be expected from PVPP excipients added as tablet disintegrants, particularly at common usage levels of 20-80 mg/tablet.

[1]Twenty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives, Geneva, WHO Tech. Rep. Ser. No. 696, 1983.

PVPP-2 11/16/94

000554

[redacted]

[redacted]

5.5 Pesticide analysis of ShanStar Cranberry extract

When submitted for pesticide screening to a third party laboratory using official EPA methodology, neither ShanStar Cranberry extract nor the juice concentrate from which it is made showed the presence of pesticides. See copy of original pesticide report in following pages.

KRUEGER
FOOD
LABORATORIES, INC.

24 Blackstone Street, Cambridge, MA 02139

TEL: (617) 876-9118
FAX: (617) 876-0572

CERTIFICATE OF ANALYSIS

Submitted by:

Date Reported: 02/04/99
Date Received: 01/21/99

Yang Wei
Cliffstar Corporation
One Cliffstar Avenue
Dunkirk, NY 14048
5459 6343 2497

Reference:

PO #:

Description	*Your Sample Number*	*Our Lab Number*
Cranberry Solids Powder	Shanstar Prod.	46028

ORGANOCHLORINE PESTICIDE SCREEN	none detected
ORGANOPHOSPHOROUS PESTICIDE SCREEN	none detected
n-METHYL CARBAMATES PESTICIDE SCREEN	none detected

000556

By:
FD

[redacted]

46028 (2/4/99)

[redacted]

KRUEGER
FOOD
LABORATORIES, INC.

24 Blackstone Street, Cambridge, MA 02139

TEL: (617) 876-9118
FAX: (617) 876-0572

CERTIFICATE OF ANALYSIS

Submitted by:

Date Reported: 02/04/99
Date Received: 01/21/99

Yang Wei
Cliffstar Corporation
One Cliffstar Avenue
Dunkirk, NY 14048
5459 6343 2497

Reference:

PO #:

Description	*Your Sample Number*	*Our Lab Number*
Cranberry Juice Concentrate		46027

ORGANOCHLORINE PESTICIDE SCREEN	none detected*
ORGANOPHOSPHOROUS PESTICIDE SCREEN	none detected*
n-METHYL CARBAMATES PESTICIDE SCREEN	none detected*

*All results reported on a single strength juice basis.

000557

By:

5.6 Analysis of ShanStar Cranberry for quercetin

The following pages contain a report from Alpha Chemical and Biomedical Laboratories, Inc. on the quercetin content of ShanStar Cranberry. Original HPLC curves are included. The batch of ShanStar Cranberry having the highest content of total phenolics was sent to Alpha for testing.

000558

ALPHA CHEMICAL & BIOMEDICAL LABORATORIES, INC.
a division of EUROFINS SCIENTIFIC, INC.
visit our websites at:
www.alphalabs.com ♦ www.eurofins.com

April 3, 2000

Brian Zanghi
Shanstar Biotech, Inc.; a division of Cliffstar Intl.
One Cliffstar Ave.
Dunkirk NY 14048

ALPHA Job # 6402

Sample Identification:

Alpha Sample #: 00-1256 ShanStar Cranberry Powder
00-1257 ShanStar Grape Powder
Date Received: 03-21-00, Time Received: 10:00 AM

ALPHA Method:

LC101: Sample Preparation
LC200: Bioflavonoids by High Performance Liquid Chromatography (HPLC)
LC224: Resveratrol by High Performance Liquid Chromatography (HPLC)

Results:

ALPHA Sample #	Quercetin (% wt/wt)	Sample Preparation
00-1256	0.21	Client preparation: Weighed about 50 milligrams. Added 1.0 ml DMSO, vortexed 30 seconds, centrifuged at approximately 9000 rpm for 2 minutes. Removed supernatant and saved. Repeated four more times combining all five supernatants. A total of 5 milliliters of DMSO was used. Sample was filtered through 0.45 µm PTFE filter.
00-1256 (D)	0.24	Test preparation for comparison: Weighed about 65 milligrams and added 5 milliliters of DMSO. Sonicated sample for about 40 minutes. Sample was filtered through 0.45 µm PTFE filter.
00-1256 (Week 1)	0.0646	First Preparation: Weighed about 840 milligrams and added 10 milliliters of methanol. Sonicated sample for about 40 minutes. Sample was filtered through a 0.45 µm PTFE filter.

(D) = Duplicate

000559

Beth Sersen
Chemist

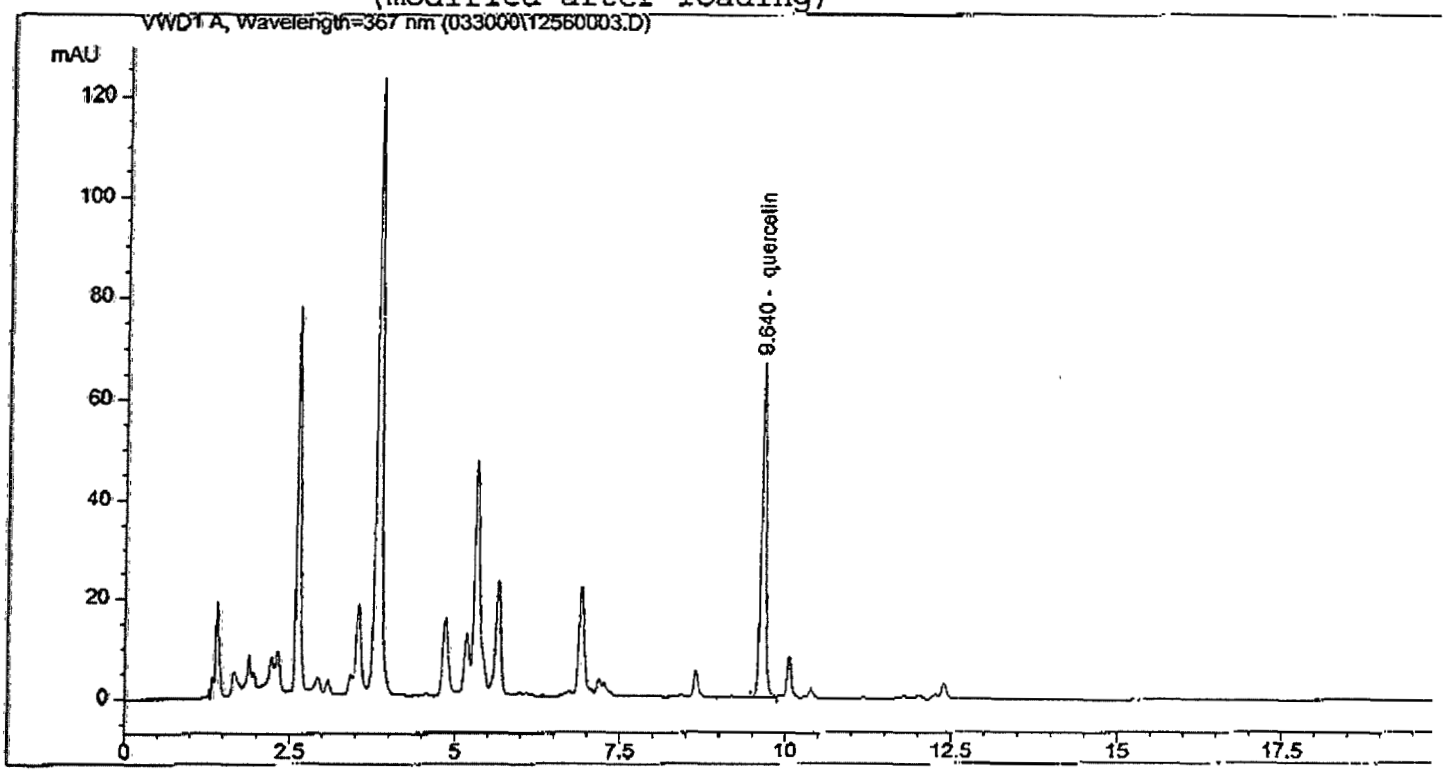
Bill J. Gannon
Quality Assurance Officer

1365 Redwood Way • Petaluma, CA 94954 USA
1-800-92-ALPHA • Tel: 707/792-7300 • Fax: 707/792-7309

(client prep.)

Injection Date	: 3/30/2000 2:50:14 PM	Seq. Line :	3
Sample Name	: sample #1256	Vial :	3
Acq. Operator	: beth sersen	Inj :	1
		Inj Volume :	5 µl

Acq. Method : C:\HPCHEM\3\METHODS\QUERCRAN.M
Last changed : 3/30/2000 3:01:14 PM by beth sersen
(modified after loading)
Analysis Method : C:\HPCHEM\3\METHODS\QUERCRAN.M
Last changed : 3/31/2000 4:07:24 PM by beth sersen
(modified after loading)



ESTD Percent Report

Sorted By	:	Signal
Calib. Data Modified	:	Friday, March 31, 2000 4:05:43 PM
Multiplier	:	1.0000
Dilution	:	1.0000
Sample Amount	:	10.13400 [mg/ml]

000560

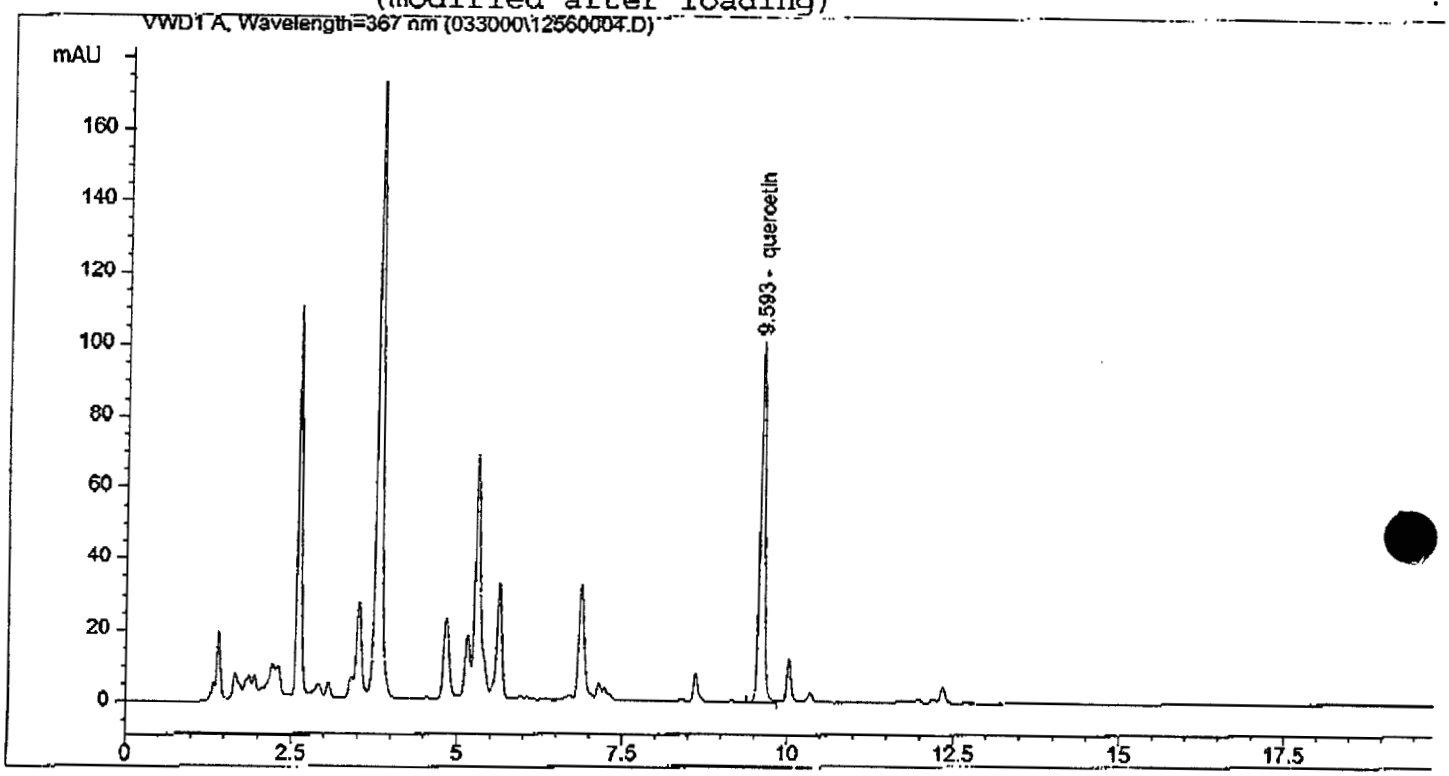
Signal 1: VWD1 A, Wavelength=367 nm

RetTime [min]	Type	Area mAU *s	Amt/Area	Amount %	Grp	Name
9.640	BV	343.85016	6.13946e-5	0.208314		quercetin
Totals :				0.208314		

Results obtained with enhanced integrator!

(test prep.)

Injection Date	: 3/30/2000 3:18:13 PM	Seq. Line :	4
Sample Name	: #1256 - dup.	Vial :	4
Acq. Operator	: beth sersen	Inj :	1
		Inj Volume :	5 µl
Acq. Method	: C:\HPCHEM\3\METHODS\QUERCRAN.M		
Last changed	: 3/30/2000 3:16:46 PM by beth sersen (modified after loading)		
Analysis Method	: C:\HPCHEM\3\METHODS\QUERCRAN.M		
Last changed	: 3/31/2000 4:07:56 PM by beth sersen (modified after loading)		



ESTD Percent Report

Sorted By	:	Signal
Calib. Data Modified	:	Friday, March 31, 2000 4:05:43 PM
Multiplier	:	1.0000
Dilution	:	1.0000
Sample Amount	:	13.21950 [mg/ml]

Signal 1: VWD1 A, Wavelength=367 nm

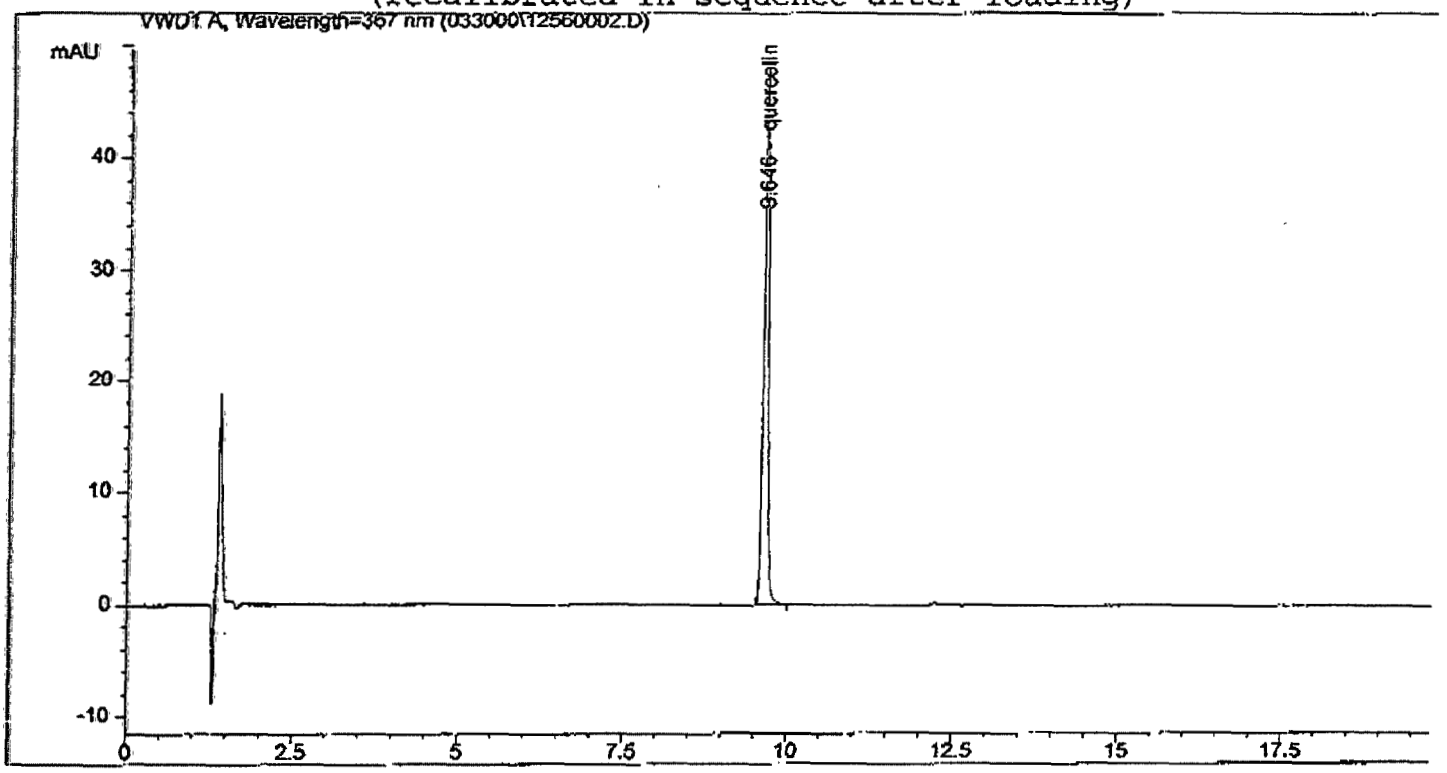
RetTime [min]	Type	Area mAU *s	Amt/Area	Amount %	Grp	Name
9.593	VV	507.32416	6.13946e-5	0.235614		quercetin
Totals :				0.235614		

000561

Results obtained with enhanced integrator!

Injection Date : 3/30/2000 2:22:15 PM Seq. Line : 2
Sample Name : ref. std. Vial : 2
Acq. Operator : beth sersen Inj : 1
Inj Volume : 5 µl

Sequence File ; C:\HPCHEM\3\SEQUENCE\QUERCRAN.S
Acq. Method : C:\HPCHEM\3\METHODS\QUERCRAN.M
Last changed : 3/30/2000 1:52:44 PM by beth sersen
Analysis Method : C:\HPCHEM\3\METHODS\QUERCRAN.M
Last changed : 3/31/2000 4:05:25 PM by beth sersen
(recalibrated in sequence after loading)



Calibration Table (after recalibration)

Calib. Data Modified : Friday, March 31, 2000 4:05:25 PM

Level 1 calibrated: Replace Response Factors,
Replace Retention Times

Signal 1: VWD1 A, Wavelength=367 nm

000562

RetTime [min]	Lvl Sig		Amount [mg/ml]	Area	Amt/Area	Ref	Grp	Name
9.646	1	1	1.44000e-2	234.94307	6.12914e-5			quercetin

Rel. Reference Window : 1.000 %
Abs. Reference Window : 0.150 min
Rel. Non-ref. Window : 1.000 %
Abs. Non-ref. Window : 0.150 min
Uncalibrated Peaks : not reported
Partial Calibration : Yes, identified peaks are recalibrated
Correct All Ret. Times: No, only for identified peaks

Curve Type : Average Response/Amount
Origin : Ignored
Weight : Equal

==========
==========
ESTD Percent Report (after recalibration)
==========

Sorted By : Signal
Calib. Data Modified : Friday, March 31, 2000 4:05:25 PM
Multiplier : 1.0000
Dilution : 1.0000
Sample Amount : 1.44000e-2 [mg/ml]

Signal 1: VWD1 A, Wavelength=367 nm

RetTime [min]	Type	Area mAU *s	Amt/Area	Amount %	Grp	Name
9.646	BB	234.94307	6.12914e-5	100.000000		quercetin

Totals : 100.000000

Results obtained with enhanced integrator!
==========
*** End of Report ***

000563

[redacted]

5.7 Estimation of myricetin and kaempferol

Replace estimates with real data as soon as we have it.

References have established quercetin, myricetin and kaempferol to be present in cranberry fruit at levels as high as ~ 250 PPM (250 mg/kg) [1,2], 27 PPM (2.7 mg/kg) [1] and 2.7 PPM (2.7 mg/kg) [1]. Based on these values and the quantitation of quercetin performed by Alpha Inc. (See Section 5.6), we believe that the ShanStar Cranberry Process concentrates the polyphenolics in cranberry fruit to ~ 10 times what is normally seen in fruits and fruit juices.

Compound	PPM in Cranberry fruit	PPM in Cranberry Juice cocktail (27%)	PPM in ShanStar Cranberry
Quercetin	250 PPM	?	2400 PPM
Myricetin	27 PPM	?	260 PPM
Kaempferol	2.7 PPM	?	26 PPM

1. Bilyk, A and Sapers, G M, Varietal differences in the quercetin, kaempferol, and myricetin contents of highbush, blueberry, cranberry and thornless blackberry fruits, Journal of Agricultural Food Chemistry, 34 (4): 585 – 588, 1986.
2. Duke, James A. 1992. Handbook of phytochemical constituents of GRAS herbs and other economic plants. Boca Raton, FL. CRC Press.

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Section 6 Supporting Data

6.1 PVP --- GRAS for use as a processing aid in the manufacture of feed for aquaculture destined for human consumption

6.2 PVP --- GRAS for use as a diluent for color additive mixtures for foods destined for human consumption

6.3 Citations from the Code for Federal regulations on approved uses of PVP and PVPP

- 6.3.A Coatings on fresh citrus fruits- PVP
- 6.3.B Diluents in color additive mixtures for use in foods- PVP
- 6.3.C Diluents in color additive mixtures for use in drugs- PVP
- 6.3.D In coatings in contact with foods - PVP
- 6.3.E In paper and paper board in contact with foods - PVP
- 6.3.F Adhesives in packaging which contacts food - PVP
- 6.3.G Clarifying agent in beverages and vinegar - PVPP
- 6.3.H Clarifying agent in beverages and vinegar - PVP

6.4 ADI review of Crospovidone, WHO Report of 27th JECFA, 1983

6.5 Summary of some original works submitted to JECFA, 1983

6.6 Review of cinical studies on PVP, Lewis Publishers 1990

000565

6.1 PVPP --- GRAS for use as a processing aid in the manufacture of feed for aquaculture destined for human consumption

See following page.

[redacted]

000566

[redacted]

INTERNATIONAL SPECIALTY PRODUCTS
1361 Alps Road Wayne NJ 07470 • Tel: 201-628-4000

May 20, 1996

Polyvinylpyrrolidone (PVP) has been affirmed as "generally recognized as safe (GRAS)" and is allowed to be used as a processing aid in the manufacture of feed for aquaculture (fish and shellfish) destined for human consumption.

The US Food and Drug Administration (FDA) recognizes that a manufacturer may conclude that a food additive is "generally recognized as safe (GRAS)" under the Federal Food, Drug, and Cosmetic Act, Sec. 201(s); 21 Code of Federal Regulations § Sec. 182. This process has been and is implemented frequently by companies, industry coalitions and trade associations. One prime example is the Flavor and Extract Manufacturers' Association which applies this procedure to achieve GRAS status for hundreds of flavor ingredients for direct use in thousands of food products.

International Specialty Products (ISP) convened an independent panel of recognized scientific and medical experts in food safety qualified by scientific training and experience to evaluate the safety of polyvinylpyrrolidone (PVP) and determine GRAS status for use as a processing aid in the manufacture of feed for aquaculture destined for human consumption.

The Expert Panel reviewed a comprehensive search of the scientific literature, including all available toxicological data, as well as the current regulatory status of PVP for use as a direct and indirect additive in human foods and beverages (US Food and Drug Administration), and the Acceptable Daily Intake (ADI) for all food uses as determined by the UN World Health Organization (WHO) / Food and Agricultural Organization (FAO) Joint Expert Committee on Food Additives (JECFA). In addition, the Expert Panel reviewed the manufacturing process and specifications for PVP as manufactured by ISP, and the anticipated exposure to humans consuming fish and shellfish fed on feed produced using PVP, as a straight per capita calculation and as a percentage of the JECFA ADI.

Based on this intensive and extensive review, the Expert Panel concluded that the available information on PVP including toxicological data and long history of safe use supported the safety of PVP for the proposed use as a processing aid for aquaculture feed.

This report, and the recognition from FDA that a manufacturer may convene a panel of experts for this process (Federal Food, Drug, and Cosmetic Act, Sec. 201(s); 21 Code of Federal Regulations § Sec. 182), supports the conclusion that ISP polyvinylpyrrolidone (PVP) is GRAS when used as a processing aid for the manufacture of aquaculture feed at levels < 5% of the formulation.

James C. Griffiths, Ph.D., Diplomate American Board of Toxicology
Manager of Toxicology, Product Stewardship Department
ISP Management Company, Inc.

6.2 PVP GRAS self affirmation for use as a diluent for color additive mixtures for foods destined for human consumption

See following page.

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INTERNATIONAL SPECIALTY PRODUCTS

1361 Alps Road Wayne NJ 07470 • Tel· 201-628-4000

March 6, 1997

Polyvinylpyrrolidone (PVP) has been affirmed as "generally recognized as safe (GRAS)" and is allowed to be used as a diluent for color additive mixtures (inks) for food use (labeling, marking, and decorating food) destined for human consumption.

The US Food and Drug Administration (FDA) recognizes that a manufacturer may conclude that a food additive is "generally recognized as safe (GRAS)" under the Federal Food, Drug, and Cosmetic Act, Sec. 201(s); 21 Code of Federal Regulations § Sec. 182. This process has been and is implemented frequently by companies, industry coalitions and trade associations. One prime example is the Flavor and Extract Manufacturers' Association which applies this procedure to achieve GRAS status for hundreds of flavor ingredients for direct use in thousands of food products.

International Specialty Products (ISP) convened an independent panel of recognized scientific and medical experts in food safety qualified by scientific training and experience to evaluate the safety of polyvinylpyrrolidone (PVP) and determine GRAS status for use as a diluent for color additive mixtures (inks) for food use (labeling, marking, and decorating food) destined for human consumption.

The Expert Panel reviewed a comprehensive search of the scientific literature, including all available toxicological data, as well as the current regulatory status of PVP for use as a direct and indirect additive in human foods and beverages (US Food and Drug Administration), and the Acceptable Daily Intake (ADI) for all food uses as determined by the UN World Health Organization (WHO) / Food and Agricultural Organization (FAO) Joint Expert Committee on Food Additives (JECFA). In addition, the Expert Panel reviewed the manufacturing process and specifications for PVP as manufactured by ISP, and the anticipated exposure to humans consuming food, to which color additive mixtures (inks) containing PVP has been used for labeling, marking, and decorating, as a straight per capita calculation and as a percentage of the JECFA ADI.

Based on this intensive and extensive review, the Expert Panel concluded that the available information on PVP including toxicological data and long history of safe use supported the safety of PVP for the proposed use as a diluent for color additive mixtures (inks) for food use (labeling, marking, and decorating food) destined for human consumption.

This report, and the recognition from FDA that a manufacturer may convene a panel of experts for this process (Federal Food, Drug, and Cosmetic Act, Sec. 201(s); 21 Code of Federal Regulations § Sec. 182), supports the conclusion that ISP polyvinylpyrrolidone (PVP) is GRAS when used as a diluent for color additive mixtures (inks) for food use (labeling, marking, and decorating food) destined for human consumption.

James C. Griffiths, Ph.D. / Diplomate American Board of Toxicology
Manager of Toxicology, Product Stewardship Department
ISP Management Company, Inc.

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6.3 Citations from the Code for Federal regulations on approved uses of PVP and PVPP

The enclosed are copies of the various approved uses of PVP and PVPP which appear in the Code of Federal Regulation (CFR). PVP is included here because it is the base polymer of PVPP and it is presented synonymously in a number of cases with PVPP. For example, PVPP is listed as a synonym to PVP when searching Toxline databases, and in the CFR, 21. 175.105, p 147 to 148, PVP (Section 6.3.F, here) and its polymers are approved for use. PVPP is no more than a crosslinked homopolymer of PVP.

000570

6.3.A Coatings on fresh citrus fruits-adjuvant: PVP

See following pages from Code of Federal Regulations.

000571

See p. 2

[Code of Federal Regulations]
[Title 21, Volume 3, Parts 170 to 199]
[Revised as of April 1, 1998]
From the U.S. Government Printing Office via GPO Access
[CITE: 21CFR172.210]

[Page 35-36]

TITLE 21--FOOD AND DRUGS

CHAPTER I--FOOD AND DRUG ADMINISTRATION, DEPARTMENT OF HEALTH AND HUMAN SERVICES (

PART 172--FOOD ADDITIVES PERMITTED FOR DIRECT ADDITION TO FOOD FOR HUMAN CONSUMPTI

Subpart C--Coatings, Films and Related Substances

Sec. 172.210 Coatings on fresh citrus fruit.

Coatings may be applied to fresh citrus fruit for protection of the fruit in accordance with the following conditions:

(a) The coating is applied in the minimum amount required to accomplish the intended effect.

(b) The coating may be formulated from the following components, each used in the minimum quantity required to accomplish the intended effect:

(1) Substances generally recognized as safe for the purpose or previously sanctioned for the purpose.

(2) One or more of the following:

Component	Limitations
Fatty acids.....	Complying with Sec. 172.860.
Oleic acid derived from tall oil fatty acids.	Complying with Sec. 172.862.
Partially hydrogenated rosin...	Catalytically hydrogenated to a maximum refractive index of 1.5012 at 100 deg.C. Color of WG or paler.
Pentaerythritol ester of maleic anhydride-modified wood rosin.	Acid number of 134-145; drop-softening point of 127 deg.C-173 deg.C; saponification number of less than 280; and a color of M or paler.
Do.....	Acid number of 176-186; drop-softening point of 110 deg.C-118 deg.C; saponification number of less than 280; and a color of M or paler.
Polyethylene glycol.....	Complying with Sec. 172.820. As a defoamer and dispersing adjuvant.
Polyhydric alcohol diesters of oxidatively refined (Gersthofen process) montan wax acids.	Complying with Sec. 178.3770 of this chapter and having a dropping point of 77 to 83 deg.C (170.6 to 181.4 deg.F), as determined by ASTM Method D566-76 (Reapproved 1982), ``Standard Test Method for Dropping Point of Lubricating Grease,'' which is incorporated by reference (copies are available from the American Society for Testing and Materials, 1916 Race St., Philadelphia, PA 19103, or available for inspection at the Office of the Federal Register, 800 North Capitol Street, NW., suite 700,

000572

[redacted]

	Washington, DC 20408) using as a solvent xylene-ethyl alcohol in a 2:1 ratio instead of toluene-ethyl alcohol in a 2:1 ratio.
Sodium lauryl sulfate.....	Complying with Sec. 172.822. As a film former.
Wood rosin.....	Color of K or paler.

(3) In lieu of the components listed in paragraph (b) (2) and (4) of this section, the following copolymer and one or more of the listed adjuvants.

Component	Limitations
Vinyl chloride-vinylidene chloride copolymer.	As an aqueous dispersion containing a minimum of 75 percent water when applied.
Polyethylene glycol.....	Complying with Sec. 172.820. As a defoamer and dispersing adjuvant.
Polyvinylpyrrolidone.....	As an adjuvant.
Potassium persulfate.....	Do.

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Propylene glycol alginate.....	Do.
Sodium decylbenzenesulfonate...	Do.

(4) In lieu of the components listed in paragraph (b) (2) and (3) of this section, the following rosin derivative and either or both of the listed adjuvants:

Component	Limitations
Calcium salt of partially dimerized rosin.	Having a maximum drop-softening point of 197 deg.C and a color of H or paler. It is prepared by reaction with not more than 7 parts hydrated lime per 100 parts of partially dimerized rosin. The partially dimerized rosin is rosin that has been dimerized by sulfuric acid catalyst to a drop-softening point of 95 deg.C to 105 deg.C and a color of WG or paler.
Petroleum naphtha.....	As adjuvant. Complying with Sec. 172.250.
Sperm oil.....	As adjuvant.

[42 FR 14491, Mar. 15, 1977; 49 FR 5747, Feb. 15, 1984, as amended at 51 FR 2693, Jan. 21, 1986; 52 FR 18911, May 20, 1987; 61 FR 14245, Apr. 1, 1996]

000573

6.3.B Diluents in color additive mixtures for use in foods: PVP

See following pages from Code of Federal Regulations.

000574

[redacted]

See p. 4

[Code of Federal Regulations]
[Title 21, Volume 1, Parts 1 to 99]
[Revised as of April 1, 1998]
From the U.S. Government Printing Office via GPO Access
[CITE: 21CFR73.1]

[Page 299-300]

TITLE 21--FOOD AND DRUGS

CHAPTER I--FOOD AND DRUG ADMINISTRATION, DEPARTMENT OF HEALTH AND HUMAN SERVICES

PART 73--LISTING OF COLOR ADDITIVES EXEMPT FROM CERTIFICATION--Table of Contents

Subpart A--Foods

Sec. 73.1 Diluents in color additive mixtures for food use exempt from certificat

The following substances may be safely used as diluents in color additive mixtures for food use exempt from certification, subject to the condition that each straight color in the mixture has been exempted from certification or, if not so exempted, is from a batch that has previously been certified and has not changed in composition since certification. If a specification for a particular diluent is not set forth in this part 73, the material shall be of a purity consistent with its intended use.

(a) General use. (1) Substances that are generally recognized as safe under the conditions set forth in section 201(s) of the act.

(2) Substances meeting the definitions and specifications set forth under subchapter B of this chapter, and which are used only as prescribed by such regulations.

(3) The following:

Substances	Definitions and specifications	Restrictions
Calcium disodium EDTA (calcium disodium ethyl-enediamine- tetraacetate).	Contains calcium disodium ethyl-enediamine-tetraacetate dihydrate (CAS Reg. No. 6766-87-6) as set forth in the Food Chemicals Codex, 3d ed., p. 50, 1981.	May be used in aqueous solutions and aqueous dispersions as a preservative and sequestrant in color additive mixtures intended only for ingested use; the color additive mixture (solution or dispersion) may contain not more than 1 percent by weight of the diluent (calculated as anhydrous calcium disodium ethyl-enediamine-tetraacetate).
Castor oil.....	As set forth in U.S.P. XVI.	Not more than 500 p.p.m. in the finished food. Labeling of color

[redacted]

000575

		additive mixtures containing castor oil shall bear adequate directions for use that will result in a food meeting this restriction.
Dioctylsodium sulfosuccinate.	As set forth in sec. 172.810 of this chapter.	Not more than 9 p.p.m. in the finished food. Labeling of color additive mixtures containing dioctylsodium sulfosuccinate shall bear adequate directions for use that will result in a food meeting this restriction.
Disodium EDTA (disodium ethyl- enediamine- tetraacetate).	Contains disodium ethyl- enediamine- tetraacetate dihydrate (CAS Reg. No. 6381-92-6) as set forth in the Food Chemicals Codex, 3d ed., p. 104, 1981.	May be used in aqueous solutions and aqueous dispersions as a preservative and sequestrant in color additive mixtures intended only for ingested use; the color additive mixture (solution or dispersion) may contain not more than 1 percent by weight of the diluent (calculated as anhydrous disodium ethyl- enediamine- tetraacetate).

Substances	Definitions and specifications	Restrictions
Alcohol, SDA-3A.....	As set forth in 26 CFR pt. 212.	No residue.
n-Butyl alcohol.....	.....	Do.
Cetyl alcohol.....	As set forth in N.F. XI.	Do.
Cyclohexane.....	.....	Do.
Ethyl cellulose.....	As set forth in sec. 172.868 of this chapter.	
Ethylene glycol monoethyl ether.	.....	Do.
Isobutyl alcohol.....	.....	Do.
Isopropyl alcohol.....	.....	Do.
Polyoxyethylene sorbitan monooleate (polysorbate 80).	As set forth in sec. 172.840 of	

000576

	this chapter.
Polyvinyl acetate.....	Molecular weight, minimum 2,000.
Polyvinylpyrrolidone.....	As set forth in sec. 173.55 of this chapter.
Rosin and rosin derivatives..	As set forth in sec. 172.615 of this chapter.
Shellac, purified.....	Food grade.....

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Substances	Definitions and specifications	Restrictions
Acetone.....	As set forth in N.F. XI.	No residue.
Alcohol, SDA-3A.....	As set forth in 26 CFR pt. 212.	Do.
Benzoin.....	As set forth in U.S.P. XVI.	
Copal, Manila.....	.....	
Ethyl acetate.....	As set forth in N.F. XI.	Do.
Ethyl cellulose.....	As set forth in sec. 172.868 of this chapter.	
Methylene chloride.....	.....	Do.
Polyvinylpyrrolidone.....	As set forth in sec. 173.55 of this chapter.	
Rosin and rosin derivatives..	As set forth in sec. 172.615 of this chapter.	
Silicon dioxide.....	As set forth in sec. 172.480 of this chapter.	Not more than 2 pct of the ink solids.
Terpene resins, natural.....	As set forth in sec. 172.615 of this chapter.	
Terpene resins, synthetic....	Polymers of <greek-a>- and <greek-b>-pinene.	

(2) Diluents in color additive mixtures for coloring shell eggs. Items listed in paragraph (a) of this section and the following, subject to the condition that there is no penetration of the color additive mixture or any of its components through the eggshell into the egg:

Alcohol, denatured, formula 23A (26 CFR part 212), Internal Revenue Service.
Damar gum (resin).
Diethylene glycol distearate.
Dioctyl sodium sulfosuccinate.
Ethyl cellulose (as identified in Sec. 172.868 of this chapter).
Ethylene glycol distearate.
Japan wax.
Limed rosin.

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Naphtha.
Pentaerythritol ester of fumaric acid-rosin adduct.
Polyethylene glycol 6000 (as identified in Sec. 172.820 of this chapter).
Polyvinyl alcohol.
Rosin and rosin derivatives (as identified in Sec. 172.820 of this chapter).

(3) Miscellaneous special uses. Items listed in paragraph (a) of this section and the following:

Substances	Definitions and specifications	Restrictions
Polyvinylpyrrolidone.....	As set forth in sec. 173.55 of this chapter.	In or as food-tablet coatings; limit, not more than 0.1 pct in the finished food; labeling of color additive mixtures containing polyvinylpyrrolidone shall bear adequate directions for use that will result in a food meeting this restriction.

[42 FR 15643, Mar. 22, 1977, as amended at 57 FR 32175, July 21, 1992]

000578

[redacted]

6.3.C Diluents in color additive mixtures for use in drugs: PVP

See following pages from Code of Federal Regulations.

[redacted]

000579

[Code of Federal Regulations]
[Title 21, Volume 1, Parts 1 to 99]
[Revised as of April 1, 1998]
From the U.S. Government Printing Office via GPO Access
[CITE: 21CFR73.1001]

[Page 314-315]

TITLE 21--FOOD AND DRUGS

CHAPTER I--FOOD AND DRUG ADMINISTRATION, DEPARTMENT OF HEALTH AND HUMAN SERVICES

PART 73--LISTING OF COLOR ADDITIVES EXEMPT FROM CERTIFICATION--Table of Contents

Subpart B--Drugs

Sec. 73.1001 Diluents in color additive mixtures for drug use exempt from certifi

The following diluents may be safely used in color additive mixtures that are exempt from certification and which are to be used for coloring drugs, subject to the condition that each straight color in the mixture has been exempted from certification or, if not so exempted, is from a batch that has previously been certified and has not changed in composition since certification. Such listing of diluents is not to be construed as superseding any of the other requirements of the Federal Food, Drug, and Cosmetic Act with respect to drugs, including new drugs. If a definition and specification for a particular diluent is not set forth in this subpart, the material shall be of a purity consistent with its intended use.

(a) Ingested drugs--(1) General use. Diluents listed in Sec. 73.1(a) and the following:

Substances	Definitions and specifications	Restrictions
Alcohol, specially denatured..	As set forth in 26 CFR, pt. 212.	As set forth in 26 CFR, pt. 211.
Cetyl alcohol.....	As set forth in N.F. XI.	
Isopropyl alcohol.....	.....	In color coatings for pharmaceutical forms, no residue.
Polyoxyethylene (20) sorbitan monostearate (Polysorbate 60).	As set forth in sec. 172.836 of this chapter.	
Polyoxyethylene (20) sorbitan tristearate (Polysorbate 65).	As set forth in sec. 172.838 of this chapter.	
Polysorbate 80.....	As set forth in sec. 172.840 of this chapter.	
Polyvinyl-pyrrolidone.....	As set forth in sec. 173.55 of this chapter.	
Sorbitan monooleate.....		
Sorbitan monostearate.....	As set forth in sec. 172.842 of this chapter.	
Sorbitan triolease.....		

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(2) Special use; inks for branding pharmaceutical forms. Items listed in paragraph (a)(1) of this section, Sec. 73.1(b)(1)(i), and the following:

Ethyl lactate
Polyoxyethylene sorbitan monolaurate (20)

(b) Externally applied drugs. Diluents listed in paragraph (a)(1) of this section and the following:

Substances	Definitions and specifications
Benzyl alcohol	As set forth in N.F. XI.
Ethyl cellulose	As set forth in Sec. 172.868 of this chapter.
Hydroxyethyl cellulose	
Hydroxypropyl cellulose	As set forth in Sec. 172.870 of this chapter.

000581

[redacted]

6.3.D Substances for use as a component of coatings: PVP

See following pages from Code of Federal Regulations.

[redacted]

See pp. 7-8

[Code of Federal Regulations]
[Title 21, Volume 3, Parts 170 to 199]
[Revised as of April 1, 1998]
From the U.S. Government Printing Office via GPO Access
[**CITE: 21CFR175.300**]

[Page 156-173]

TITLE 21--FOOD AND DRUGS

CHAPTER I--FOOD AND DRUG ADMINISTRATION, DEPARTMENT OF HEALTH AND HUMAN SERVICES (

PART 175--INDIRECT FOOD ADDITIVES: ADHESIVES AND COMPONENTS OF COATINGS--Table of

Subpart C--Substances for Use as Components of Coatings

Sec. 175.**300** Resinous and polymeric coatings.

Resinous and polymeric coatings may be safely used as the food-contact surface of articles intended for use in producing, manufacturing, packing, processing, preparing, treating, packaging, transporting, or holding food, in accordance with the following prescribed conditions:

(a) The coating is applied as a continuous film or enamel over a metal substrate, or the coating is intended for repeated food-contact use and is applied to any suitable substrate as a continuous film or enamel that serves as a functional barrier between the food and the substrate. The coating is characterized by one or more of the following descriptions:

(1) Coatings cured by oxidation.

(2) Coatings cured by polymerization, condensation, and/or cross-linking without oxidation.

(3) Coatings prepared from prepolymerized substances.

(b) The coatings are formulated from optional substances that may include:

(1) Substances generally recognized as safe in food.

(2) Substances the use of which is permitted by regulations in this part or which are permitted by prior sanction or approval and employed under the specific conditions, if any, of the prior sanction or approval.

(3) Any substance employed in the production of resinous and polymeric coatings that is the subject of a regulation in subchapter B of this chapter and conforms with any specification in such regulation. Substances named in this paragraph (b)(3) and further identified as required:

(i) Drying oils, including the triglycerides or fatty acids derived therefrom:

Beechnut.
Candlenut.
Castor (including dehydrated).
Chinawood (tung).
Coconut.
Corn.
Cottonseed.
Fish (refined).
Hempseed.
Linseed.
Oiticica.
Perilla.
Poppyseed.
Pumpkinseed.
Safflower.

[redacted]

[[Page 160]]

linking agent at a level not to exceed 10 percent by weight of the cured coating, provided that the cured coating only contacts food containing not more than 8 percent alcohol.
Meta-Xylylenediamine (1,3-benzenedimethanamine, CAS Reg. No. 1477-55-0), for use only in coatings at a level not to exceed 3 percent by weight of the resin when such coatings are intended for repeated use in contact with foods only of the types identified in paragraph (d) of this section, table 1, under Types I, II, and III, under conditions of use C, D, E or F as described in table 2 of paragraph (d) of this section; or when such coatings are intended for repeated use in contact with foods of the types identified in paragraph (d) of this section, table 1, under Types V, VI, VII, and VIII, under conditions of use E or F as described in table 2 of paragraph (d) of this section. Use shall be limited to coatings for tanks of capacity greater than 530,000 gallons.
Para-Xylylenediamine (1,4 benzenedimethanamine, CAS Reg. No. 539-48-0), for use only in coatings at a level not to exceed 0.6 percent by weight of the resin when such coatings are intended for repeated use in contact with foods only of the types identified in paragraph (d) of this section, table 1, under Types I, II, III, under conditions of use C, D, E, or F as described in table 2 of paragraph (d) of this section; or when such coatings are intended for repeated use in contact with foods of the types identified in paragraph (d) of this section, table 1, under Types V, VI, VII, and VIII, under conditions of use E and F as described in table 2 of paragraph (d) of this section. Use shall be limited to coatings for tanks of capacity greater than 530,000 gallons.

(c) Adjuncts for epoxy resins:

Aluminum butylate.
Benzoic acid, for use as a component in epoxy resins for coatings not exceeding a coating weight of 4 milligrams per square inch and that are intended for contact under conditions of use D, E, F or G described in table 2 of paragraph (d) of this section with alcoholic beverages containing less than 8 percent alcohol.
Polyamides from dimerized vegetable oils and the amine catalysts listed in paragraph (b)(3)(viii)(b) of this section, as the basic polymer.
Silane coupled silica, prepared from the reaction of microcrystalline quartz with N-beta-(N-vinylbenzylamino) ethyl-gamma-aminopropyltrimethoxy silane, monohydrogen chloride, for use only in coatings intended for repeated use in contact with foods only of the types identified in paragraph (d) of this section, table 1, under Category I and Category VIII, at temperatures not exceeding 88 deg.C (190 deg.F).
Succinic anhydride, for use as a component in epoxy resins for coatings not exceeding a coating weight of 4 milligrams per square inch, and that are intended for contact under conditions of use D, E, F or G described in table 2 of paragraph (d) of this section with alcoholic beverages containing less than 8 percent alcohol.

(ix) Coumarone-indene resin, as the basic polymer.
(x) Petroleum hydrocarbon resin (cyclopentadiene type), as the basic polymer.
(xi) Terpene resins, as the basic polymer, from one or more of the following:

Dipentene.
Hydrogenated dipentene resin (CAS Reg. No. 106168-39-2). For use only with coatings in contact with acidic and aqueous foods.
Hydrogenated-beta-pinene-alpha-pinene-dipentene copolymer resin (CAS Reg. No. 106168-37-0). For use only with coatings in contact with acidic and aqueous foods.
<greek-a>-Pinene.

<greek-b>-Pinene.

(xii) Urea-formaldehyde, resins and their curing catalyst:
(a) Urea-formaldehyde resins, as the basic polymer:

Urea-formaldehyde.
Urea-formaldehyde chemically modified with methyl, ethyl, propyl, isopropyl, butyl, or isobutyl alcohol.
Urea-formaldehyde chemically modified with one or more of the amine catalysts listed in paragraph (b)(3)(viii)(b) of this section.

(b) Curing (cross-linking) catalyst for urea-formaldehyde resins:

Dodecyl benzenesulfonic acid (C.A. Registry No. 27176-87-0).

(xiii) Triazine-formaldehyde resins and their curing catalyst:
(a) Triazine-formaldehyde resins, as the basic polymer:

Benzoguanamine-formaldehyde.
Melamine-formaldehyde.
Melamine-formaldehyde chemically modified with one or more of the following amine catalysts:
Amine catalysts listed in paragraph (b)(3)(viii)(b) of this section.
Dimethylamine-2-methyl-1-propanol.
Methylpropanolamine.
Triethanolamine.
Melamine-formaldehyde chemically modified with methyl, ethyl, propyl, isopropyl, butyl, or isobutyl alcohol.

(b) Curing (cross-linking) catalyst for triazine-formaldehyde resins:

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Dodecyl benzenesulfonic acid (C.A. Registry No. 27176-87-0).

(xiv) Modifiers (for oils and alkyds, including polyesters), as the basic polymer:

Butyl methacrylate.
Cyclopentadiene.
Methyl, ethyl, butyl, or octyl esters of acrylic acid.
Methyl methacrylate.
Styrene.
Vinyl toluene.

(xv) Vinyl resinous substance, as the basic polymers:

Polyvinyl acetate.
Polyvinyl alcohol.
Polyvinyl butyral.
Polyvinyl chloride.
Polyvinyl formal.
Polyvinylidene chloride.
Polyvinyl pyrrolidone.
Polyvinyl stearate.
Vinyl chloride-acetate-2,3-epoxypropyl methacrylate copolymers containing not more than 10 weight percent of total polymer units derived from 2,3-epoxypropyl methacrylate and not more than 0.1 weight percent of unreacted 2,3-epoxypropyl methacrylate monomer for use in coatings for containers.
Vinyl chloride-acetate, hydroxyl-modified copolymer.

6.3.E Substances for use only as components of paper and paper board in contact with foods: PVP

See following pages from Code of Federal Regulations.

[redacted]

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[redacted]

See pp. 25, 26, 32, 38-44

[Code of Federal Regulations]
[Title 21, Volume 3, Parts 170 to 199]
[Revised as of April 1, 1998]
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[Page 180-205]

TITLE 21--FOOD AND DRUGS

CHAPTER I--FOOD AND DRUG ADMINISTRATION, DEPARTMENT OF HEALTH AND HUMAN SERVICES (

PART 176--INDIRECT FOOD ADDITIVES: PAPER AND PAPERBOARD COMPONENTS--Table of Conte

Subpart B--Substances for Use Only as Components of Paper and Paperboard

Sec. 176.170 Components of paper and paperboard in contact with aqueous and fatty

Substances identified in this section may be safely used as components of the uncoated or coated food-contact surface of paper and paperboard intended for use in producing, manufacturing, packaging, processing, preparing, treating, packing, transporting, or holding aqueous and fatty foods, subject to the provisions of this section. Components of paper and paperboard in contact with dry food of the type identified under Type VIII of table 1 in paragraph (c) of this section are subject to the provisions of Sec. 176.180.

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(a) Substances identified in paragraph (a) (1) through (5) of this section may be used as components of the food-contact surface of paper and paperboard. Paper and paperboard products shall be exempted from compliance with the extractives limitations prescribed in paragraph (c) of this section: Provided, That the components of the food-contact surface consist entirely of one or more of the substances identified in this paragraph: And provided further, That if the paper or paperboard when extracted under the conditions prescribed in paragraph (c) of this section exceeds the limitations on extractives contained in paragraph (c) of this section, information shall be available from manufacturing records from which it is possible to determine that only substances identified in this paragraph (a) are present in the food-contact surface of such paper or paperboard.

(1) Substances generally recognized as safe in food.

(2) Substances generally recognized as safe for their intended use in paper and paperboard products used in food packaging.

(3) Substances used in accordance with a prior sanction or approval.

(4) Substances that by regulation in parts 170 through 189 of this chapter may be safely used without extractives limitations as components of the uncoated or coated food-contact surface of paper and paperboard in contact with aqueous or fatty food, subject to the provisions of such regulation.

(5) Substances identified in this paragraph, as follows:

List of Substances	Limitations
Acetyl peroxide.....	For use only as polymerization catalyst.
Acrylamide-methacrylic acid-maleic anhydride copolymers containing not more than 0.2 percent of residual acrylamide monomer and having an average nitrogen content of 14.9	For use only as a retention aid employed prior to the sheet-forming operation in the manufacture of paper and paperboard in such an amount

	filler, sizing, and similar formulations added prior to the heat drying step in the manufacture of paper and paperboard and limited to use at a level between 0.05 and 0.15 percent by weight of the formulation.
Undecafluorocyclohexanemethanol ester mixture of dihydrogen phosphate, compound with 2,2' iminodiethanol (1:1); hydrogen phosphate, compound with 2,2'-iminodiethanol (1:1); and P,P'-dihydrogen pyrophosphate, compound with 2,2'-iminodiethanol (1:2); where the ester mixture has a fluorine content of 48.3 pct to 53.1 pct as determined on a solids basis.	For use only as an oil repellent at a level not to exceed 0.087 lb (0.046 lb of fluorine) per 1,000 ft 2 of treated paper or paperboard, as determined by analysis for total fluorine in the treated paper or paperboard without correction for any fluorine which might be present in the untreated paper or paperboard, when such paper or paperboard is used in contact with food only of the types identified in paragraph (c) of this section, table 1, under Types IVA, V, VIIA, VIII, and IX, and under the conditions of use B through G described in table 2 of paragraph (c) of this section.
Viscose rayon fibers.....	
Wax, petroleum.....	Complying with Sec. 178.3710 of this chapter.
Xanthan gum, conforming to the identity and specifications prescribed in Sec. 172.695 of this chapter, except that the residual isopropyl alcohol shall not exceed 6,000 parts per million.	For use only at a maximum level of 0.125 percent by weight of finished paper as a suspension aid or stabilizer for aqueous pigment slurries employed in the manufacture of paper and paperboard.
Xylene sulfonic acid-formaldehyde condensate, sodium salt.	For use only as an adjuvant to control pulp absorbency and pitch content in the manufacture of paper and paperboard prior to the sheet-forming operation.
Zeolite Na-A (CAS Reg. No. 68989-22-0).	For use as a pigment extender at levels not to exceed 5.4 percent by weight of the finished paper and paperboard.
Zinc formaldehyde sulfoxylate.....	For use only as polymerization catalyst.
Zinc octoate.....	
Zirconium oxide.....	For use only as a component of waterproof coatings where the zirconium oxide is present at a level not to exceed 1 percent by weight of the dry paper or paperboard fiber and where the zirconium oxide is produced by hydrolysis of zirconium acetate.

(b) Substances identified in paragraphs (b) (1) and (2) of this

section may be used as components of the food-contact surface of paper and paperboard, provided that the food-contact surface of the paper or paperboard complies with the extractives limitations prescribed in paragraph (c) of this section.

(1) Substances identified in Sec. 175.300(b)(3) of this chapter with the

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exception of those identified in paragraphs (b)(3) (v), (xv), (xx), (xxvi), (xxxi), and (xxxii) of that section and paragraph (a) of this section.

(2) Substances identified in this paragraph (b)(2) follow:

List of substances	Limitations
Acrylamide copolymerized with ethyl acrylate and/or stryene and/or methacrylic acid, subsequently reacted with formaldehyde and butyl alcohol.	
Acrylamide copolymerized with ethylene and vinyl chloride in such a manner that the finished copolymers have a minimum weight average molecular weight of 30,000 and contain not more than 3.5 weight percent of total polymer units derived from acrylamide, and in such a manner that the acrylamide portion may or may not be subsequently partially hydrolyzed.	For use only as coatings or components of coatings.
Acrylic and modified acrylic polymers..	Complying with Sec. 177.1010 of this chapter.
Acrylic copolymers produced by copolymerizing 2 or more of the acrylate monomers butyl acrylate, ethyl acrylate, ethyl methacrylate, methyl acrylate, methyl methacrylate, and n-propyl methacrylate, or produced by copolymerizing one or more of such acrylate monomers together with one or more of the monomers acrylic acid, acrylonitrile, butadiene, 2-ethyl-hexyl acrylate, fumaric acid, glycidyl methacrylate, n-hexyl-methacrylate, itaconic acid, methacrylic acid, styrene, vinyl acetate, vinyl chloride, and vinylidene chloride. The finished copolymers shall contain at least 50 weight percent of polymer units derived from one or more of the monomers butyl acrylate, ethyl acrylate, ethyl methacrylate, methyl acrylate, methyl methacrylate, and n-propyl methacrylate; and shall contain not more than 5 weight percent of total polymer units derived from acrylic acid, fumaric acid, glycidyl methacrylate, n-hexyl methacrylate, itaconic acid, and methacrylic acid. The provision limiting the finished acrylic copolymers to not more than 5 units derived from acrylic acid, fumaric acid, glycidyl methacrylate, n-	

172.880(b) of this chapter when subjected to the analytical procedure described in Sec. 172.886(b) of this chapter, modified as follows: Treat the product as in the first paragraph under ``Procedure'' in Sec. 172.250(b)(3) of this chapter. Then proceed with Sec. 172.886(b) of this chapter, starting with the paragraph commencing with ``Promptly complete transfer of the sample * * *''.	
Polyester resin formed by the reaction of the methyl ester of rosin, phthalic anhydride, maleic anhydride and ethylene glycol, such that the polyester resin has an acid number of 4 to 11, a drop-softening point of 70 deg.C-92 deg.C., and a color of K or paler.	
Polyester resin produced by reacting the acid groups in montan wax with ethylene glycol.	
Polyethylene, oxidized.....	Complying with Sec. 177.1620 of this chapter.
Polyethylene reacted with maleic anhydride such that the modified polyethylene has a saponification number not in excess of 6 after Soxhlet extraction for 24 hours with anhydrous ethyl alcohol.	
Polyoxyethylated (40 moles) tallow alcohol sulfate, solium salt.	Not to exceed 300 p.p.m. in finished coated paper or paperboard.
Polyoxypropylene-polyoxyethylene block polymers (minimum molecular weight 6,800).	
Polyvinyl acetate.....	
Polyvinyl alcohol (minimum viscosity of 4% aqueous solution at 20 deg.C. of 4 centipoises).	
Polyvinyl butyral.....	
Polyvinyl formal.....	
Polyvinylidene chloride.....	
Polyvinyl pyrrolidone.....	
Polyvinyl stearate.....	
Propylene glycol mono- and diesters of fats and fatty acids.	
Siloxanes (silicones), dimethyl, isopropyl methyl, methyl 1-methyl-C<INF>9-<INF>49-alkyl (CAS Reg. No. 144635-08-5)<INF>.	For use only as a component of polyolefin coatings with Sec. 177.1520 of this chapter at a level not to exceed 3 percent by weight. The finished coating will be used only for paper and paperboard that contact food of types VI-A and VI-B of table 1 in paragraph (c) of this section, and under conditions of use C, D, and E, as described in table 2 in paragraph (c) of this section, with a maximum hot fill temperature of 200 deg.F (94 deg.C).
Siloxanes and silicones; platinum-	Platinum content not to exceed

acrylamide acrylic acid, acrylonitrile, butyl acrylate, butyl methacrylate ethyl acrylate, ethyl methacrylate, fumaric acid, itaconic acid, methacrylic acid, methyl acrylate, methyl methacrylate, octadecyl methacrylate, propyl acrylate, propyl methacrylate, vinyl chloride and vinyl sulfonic acid. The finished copolymers shall contain at least 50 weight percent of polymer units derived from vinylidene chloride; and shall contain no more than 5 weight percent of total polymer units derived from acrylamide, acrylic acid, fumaric acid, itaconic acid, methacrylic acid, octadecyl methacrylate, and vinyl sulfonic acid.

Colorants:	
Aluminum	For use as a colorant only.
Aluminum hydrate	Do.
Aluminum and potassium silicate (mica).	Do.
Aluminum mono-, di-, and tristearate.	Do.
Aluminum silicate (China clay)	Do.
Barium sulfate	Do.
Bentonite	Do.
Bentonite, modified with dimethyldioctadecylammonium ion.	Do.
Burnt umber	Do.
Calcium carbonate	Do.
Calcium silicate	Do.
Calcium sulfate	Do.
Carbon black (channel process)	Do.
Cobalt aluminate	Do.
Diatomaceous earth	Do.
Iron oxides	Do.
Magnesium oxide	Do.
Magnesium silicate (talc)	Do.
Phthalocyanine blue (C.I. pigment blue 15, 15:1, 15:2, 15:3, and 15:4; C.I. No. 74160; CAS Reg. No. 147-14-8).	Do.
Raw sienna	Do.
Silica	Do.
Tartrazine lake (certified FD&C Yellow No. 5 only).	Do.
Titanium dioxide	Do.
Titanium dioxide-barium sulfate	Do.
Titanium dioxide-magnesium silicate	Do.
Zinc carbonate	Do.

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Zinc oxide	Do.

(c) The food-contact surface of the paper and paperboard in the finished form in which it is to contact food, when extracted with the solvent or solvents characterizing the type of food, and under conditions of time and temperature characterizing the conditions of its intended use as determined from tables 1 and 2 of this paragraph, shall

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yield net chloroform-soluble extractives (corrected for wax, petrolatum, mineral oil and zinc extractives as zinc oleate) not to exceed 0.5 milligram per square inch of food-contact surface as determined by the methods described in paragraph (d) of this section.

Table 1--Types of Raw and Processed Foods

I. Nonacid, aqueous products; may contain salt or sugar or both (pH above 5.0).
II. Acid, aqueous products; may contain salt or sugar or both, and including oil-in-water emulsions of low- or high-fat content.
III. Aqueous, acid or nonacid products containing free oil or fat; may contain salt, and including water-in-oil emulsions of low- or high-fat content.
IV. Dairy products and *COM001*modifications:
  A. Water-in-oil emulsions, high- or low-fat.
  B. Oil-in-water emulsions, high- or low-fat.
V. Low-moisture fats and oil.
VI. Beverages:
  A. Containing up to 8 percent of alcohol.
  B. Nonalcoholic.
  C. Containing more than 8 percent alcohol.
VII. Bakery products other than those included under Types VIII or IX of this table:
  A. Moist bakery products with surface containing free fat or oil.
  B. Moist bakery products with surface containing no free fat or oil.
VIII. Dry solids with the surface containing no free fat or oil (no end test required).
IX. Dry solids with the surface containing free fat or oil.

Table 2--Test Procedures with Time Temperature Conditions for Determining Amount Contact Surface of Uncoated or Coated Paper and Paperboard, Using Solvents Sim Beverages

Condition of use	Types of food (see table 1)	Food-simulatin: Water: Time and temperature	Food-simulatin: Heptane \1\: Time and temperature
A. High temperature heat-sterilized (e.g., over 212 deg.F).	I, IV-B, VII-B. III, IV-A, VII-A.	250 deg.F, 2 hr. .....do.....	150 deg.F, 2 hr.
B. Boiling water sterilized.	II, VII-B..... III, VII-A.....	212 deg.F, 30 min. .....do.....	..... 120 deg.F, 30 min.
C. Hot filled or pasteurized above 150 deg.F.	II, IV-B, VII-B III, IV-A, VII-A. V, IX.....	Fill boiling, cool to 100 deg.F. .....do.....	..... 120 deg.F, 15 min. .....do.....
D. Hot filled or pasteurized below 150 deg.F.	II, IV-B, VI-B, VII-B..... III, IV-A, VII-A. V, IX..... VI-A..... VI-C.....	..... 150 deg.F, 2 hr. .....do.....	.... 100 deg.F, 30 min. .....do..... ....
E. Room temperature filled and stored (no thermal treatment in the container).	I, II, IV-B, VI-B, VII-B. III, IV-A, VII-	120 deg.F, 24 hr. .....do.....	..... 70 deg.F, 30 min.

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	A.	.....	.....do.....	1
	V, IX.....	.....	.....	
	VI-A.....	.....	.....	.
	VI-C.....			
F. Refrigerated storage (no thermal treatment in the container).	III, IV-A, VII-A.	70 deg.F, 48 hr.	70 deg.F, 30 min.	.
	I, II, IV-B, VI-B, VII-B.	.....do.....	.....	7
	VI-A.....	.....	.....	.
	VI-C.....			

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G. Frozen storage (no thermal treatment in the container).	I, II, IV-B, VII-B.	70 deg.F, 24 hr.	.....	.
	III, VII-A.....	.....do.....	70 deg.F, 30 min.	.
H. Frozen or refrigerated storage: Ready-prepared foods intended to be reheated in container at time of use:.				
1. Aqueous or oil-in-water emulsion of high- or low-fat.	I, II, IV-B, VII-B.	212 deg.F, 30 min.		
2. Aqueous, high- or low-free oil or fat.	III, IV-A, VII-A, IX.	.....do.....	120 deg.F, 30 min.	

\1\ Heptane extractability results must be divided by a factor of five in arriving food product having water-in-oil emulsion or free oil or fat. Heptane food-simul in the case of wax-polymer blend coatings for corrugated paperboard containers i packaging of iced meat, iced fish, and iced poultry.

(d) Analytical methods--(1) Selection of extractability conditions. First ascertain the type of food product (table 1, paragraph (c) of this section) that is being packed commercially in the paper or paperboard and the normal conditions of thermal treatment used in packaging the type of food involved. Using table 2, paragraph (c) of this section, select the food-simulating solvent or solvents and the time-temperature exaggerations of the paper or paperboard use conditions. Having selected the appropriate food-simulating solvent or solvents and the time-temperature exaggeration over normal use, follow the applicable extraction procedure.

(2) Reagents--(i) Water. All water used in extraction procedures should be freshly demineralized (deionized) distilled water.

(ii) n-Heptane. Reagent grade, freshly redistilled before use, using only material boiling at 208 deg.F.

(iii) Alcohol. 8 or 50 percent (by volume), prepared from undenatured 95 percent ethyl alcohol diluted with demineralized (deionized) distilled water.

(iv) Chloroform. Reagent grade, freshly redistilled before use, or a grade having an established consistently low blank.

(3) Selection of test method. Paper or paperboard ready for use in packaging shall be tested by use of the extraction cell described in ``Official Methods of Analysis of the Association of Official Analytical Chemists,'' 13th Ed. (1980), sections 21.010-21.015, under ``Exposing Flexible Barrier Materials for Extraction,'' which is incorporated by reference (copies may be obtained from the Association of Official Analytical Chemists International, 481 North Frederick Ave., suite 500, Gaithersburg, MD 20877-2504, or may be examined at the Office of the Federal Register, 800 North Capitol Street, NW., suite 700, Washington, DC 20408); also described in ASTM method F34-76 (Reapproved 1980), ``Standard Test Method for Liquid Extraction of Flexible Barrier Materials,'' which is incorporated by reference (copies may be obtained

from the American Society for Testing Materials, 1916 Race St., Philadelphia, PA 19103, or may be examined at the Office of the Federal Register, 800 North Capitol Street, NW., suite 700, Washington, DC 20408), except that formed paper and paperboard products may be tested in the container by adapting the in-container methods described in Sec. 175.300(e) of this chapter. Formed paper and paperboard products such as containers and lids, that cannot be tested satisfactorily by any of the above methods may be tested in specially designed extraction equipment, usually consisting of clamping devices that fit the closure or container so that the food-contact surface can be tested, or, if flat samples can be cut from the formed paper

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or paperboard products without destroying the integrity of the food-contact surface, they may be tested by adapting the following ``sandwich'' method:

(i) Apparatus. (a) Thermostated (^{plus-minus}1.0 deg.F) water bath, variable between 70 deg.F and 120 deg.F water bath cover capable of holding at least one 800-milliliter beaker partially submersed in bath.

(b) Analytical balance sensitive to 0.1 milligram with an approximate capacity of 100 grams.

(c) Tongs.

(d) Hood and hot-plate facilities.

(e) Forced draft oven.

For each extraction, the following additional apparatus is necessary:

(f) One No. 2 paper clip.

(g) One 800-milliliter beaker with watch-glass cover.

(h) One 250-milliliter beaker.

(i) Five 2\1/2\-inch-square aluminum screens (standard aluminum window screening is acceptable).

(j) One wire capable of supporting sample stack.

(ii) Procedure. (a) For each extraction, accurately cut eight 2\1/2\-inch-square samples from the formed paper or paperboard product to be tested.

(b) Carefully stack the eight 2\1/2\-inch-square samples and the five 2\1/2\-inch-square aluminum screens in sandwich form such that the food-contact side of each sample is always next to an aluminum screen, as follows: Screen, sample, sample, screen, sample, sample, screen, etc. Clip the sandwich together carefully with a No. 2 paper clip, leaving just enough space at the top to slip a wire through.

(c) Place an 800-milliliter beaker containing 100-milliliters of the appropriate food-simulating solvent into the constant temperature bath, cover with a watch glass and condition at the desired temperature.

(d) After conditioning, carefully lower the sample sandwich with tongs into the beaker.

(e) At the end of the extraction period, using the tongs, carefully lift out the sample sandwich and hang it over the beaker with the wire.

(f) After draining, pour the food-simulating solvent solution into a tared 250-milliliter beaker. Rinse the 800-milliliter beaker three times, using a total of not more than 50 milliliters of the required solvent.

(g) Determine total nonvolatile extractives in accordance with paragraph (d)(5) of this section.

(4) Selection of samples. Quadruplicate samples should be tested, using for each replicate sample the number of cups, containers, or preformed or converted products nearest to an area of 100 square inches.

(5) Determination of amount of extractives--(i) Total residues. At the end of the exposure period, remove the test container or test cell from the oven and combine the solvent for each replicate in a clean Pyrex (or equivalent) flask or beaker being sure to rinse the test

container or cell with a small quantity of clean solvent. Evaporate the food-simulating solvents to about 100 milliliters in the flask or beaker, and transfer to a clean, tared evaporating dish (platinum or Pyrex), washing the flask three times with small portions of solvent used in the extraction procedure, and evaporate to a few milliliters on a nonsparking, low-temperature hotplate. The last few milliliters should be evaporated in an oven maintained at a temperature of approximately 221 deg.F. Cool the evaporating dish in a desiccator for 30 minutes and weigh the residue to the nearest 0.1 milligram, (e). Calculate the extractives in milligrams per square inch of the container or sheeted paper or paperboard surface.

(a) Water and 8- and 50-percent alcohol. Milligrams extractives per square inch=(e)/(s).

(b) Heptane. Milligrams extractives per square inch=(e)/(s)(F)

where:

e=Milligrams extractives per sample tested.
s=Surface area tested, in square inches.
F=Five, the ratio of the amount of extractives removed by heptane under exaggerated time-temperature test conditions compared to the amount extracted by a fat or oil under exaggerated conditions of thermal sterilization and use.
e'=Chloroform-soluble extractives residue.
ee'=Corrected chloroform-soluble extractives residue.
e' or ee' is substituted for e in the above equations when necessary.

If when calculated by the equations in paragraph (d)(5)(i) (a) and (b) of this section, the extractives in milligrams

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per square inch exceeds the limitations prescribed in paragraph (c) of this section, proceed to paragraph (d)(5)(ii) of this section (method for determining the amount of chloroform-soluble extractives residues).

(ii) Chloroform-soluble extractives residue. Add 50 milliliters of chloroform (freshly distilled reagent grade or a grade having an established consistently low blank) to the dried and weighed residue, (e), in the evaporating dish obtained in paragraph (d)(5)(i) of this section. Warm carefully, and filter through Whatman No. 41 filter paper (or equivalent) in a Pyrex (or equivalent) funnel, collecting the filtrate in a clean, tared evaporating dish (platinum or Pyrex). Repeat the chloroform extraction, washing the filter paper with this second portion of chloroform. Add this filtrate to the original filtrate and evaporate the total down to a few milliliters on a low-temperature hotplate. The last few milliliters should be evaporated in an oven maintained at approximately 221 deg.F. Cool the evaporating dish in a desiccator for 30 minutes and weigh to the nearest 0.1 milligram to get the chloroform-soluble extractives residue (\'). This \' is substituted for e in the equations in paragraph (d)(5)(i) (a) and (b) of this section. If the chloroform-soluble extractives in milligrams per square inch still exceeds the limitation prescribed in paragraph (c) of this section, proceed to paragraph (d)(5)(iii) of this section (method for determining corrected chloroform-soluble extractives residue).

(iii) Corrected chloroform-soluble extractives residue--(a) Correction for zinc extractives. Ash the residue in the evaporating dish by heating gently over a Meker-type burner to destroy organic matter and hold at red heat for about 1 minute. Cool in the air for 3 minutes, and place the evaporating dish in the desiccator for 30 minutes and weigh to the nearest 0.1 milligram. Analyze this ash for zinc by standard Association of Official Agricultural Chemists methods or equivalent. Calculate the zinc in the ash as zinc oleate, and subtract from the weight of chloroform-soluble extractives residue (\') to obtain the zinc-

corrected chloroform-soluble extractives residue (e'). This e' is substituted for e in the equations in paragraph (d)(5)(i) (a) and (b) of this section.

(b) Correction for wax, petrolatum, and mineral oil--(1) Apparatus. Standard 10 millimeter inside diameter x 60 centimeter chromatographic column (or standard 50-milliliter buret with an inside diameter of 10-11 millimeters) with a stopcock of glass, perfluorocarbon resin, or equivalent material. The column (or buret) may be optionally equipped with an integral coarse, fritted glass disc and the top of the column (or buret) may be optionally fitted with a 100-millimeter solvent reservoir.

(2) Preparation of column. Place a snug pledget of fine glass wool in the bottom of the column (or buret) if the column (or buret) is not equipped with integral coarse, fritted glass disc. Overlay the glass wool pledget (or fritted glass disc) with a 15-20 millimeter deep layer of fine sand. Measure in a graduated cylinder 15 milliliters of chromatographic grade aluminum oxide (80-200 mesh) that has been tightly settled by tapping the cylinder. Transfer the aluminum oxide to the chromatographic tube, tapping the tube during and after the transfer so as to tightly settle the aluminum oxide. Overlay the layer of aluminum oxide with a 1.0-1.5 centimeter deep layer of anhydrous sodium sulfate and on top of this place an 8-10 millimeter thick plug of fine glass wool. Next carefully add about 25 milliliters of heptane to the column with stopcock open, and allow the heptane to pass through the column until the top level of the liquid just passes into the top glass wool plug in the column, and close stopcock.

(3) Chromatographing of sample extract--(i) For chloroform residues weighing 0.5 gram or less. To the dried and weighed chloroform-soluble extract residue in the evaporating dish, obtained in paragraph (d)(5)(ii) of this section, add 20 milliliters of heptane and stir. If necessary, heat carefully to dissolve the residue. Additional heptane not to exceed a total volume of 50 milliliters may be used if necessary to complete dissolving. Cool to room temperature. (If solution becomes cloudy, use the procedure in paragraph

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(d)(5)(iii)(b)(3)(ii) of this section to obtain an aliquot of heptane solution calculated to contain 0.1-0.5 gram of chloroform-soluble extract residue.) Transfer the clear liquid solution to the column (or buret). Rinse the dish with 10 millimeters of additional heptane and add to column. Allow the liquid to pass through the column into a clean, tared evaporating dish (platinum or Pyrex) at a dropwise rate of about 2 milliliters per minute until the liquid surface reaches the top glass wool plug; then close the stopcock temporarily. Rinse the Pyrex flask which contained the filtrate with an additional 10-15 milliliters of heptane and add to the column. Wash (elute) the column with more heptane collecting about 100 milliliters of total eluate including that already collected in the evaporating dish. Evaporate the combined eluate in the evaporating dish to dryness on a steam bath. Dry the residue for 15 minutes in an oven maintained at a temperature of approximately 221 deg.F. Cool the evaporating dish in a desiccator for 30 minutes and weigh the residue to the nearest 0.1 milligram. Subtract the weight of the residue from the weight of chloroform-soluble extractives residue (') to obtain the wax-, petrolatum-, and mineral oil-corrected chloroform-soluble extractives residue (e'). This e' is substituted for e in the equations in paragraph (d)(5)(i) (a) and (b) of this section.

(ii) For chloroform residues weighing more than 0.5 gram. Redissolve the dried and weighed chloroform-soluble extract residue as described in paragraph (d)(5)(iii)(b)(3)(i) of this section using proportionately larger quantities of heptane. Transfer the heptane solution to an appropriate-sized volumetric flask (i.e., a 250-milliliter flask for about 2.5 grams of residue) and adjust to volume with additional heptane. Pipette out an aliquot (about 50 milliliters) calculated to

000597

contain 0.1-0.5 gram of the chloroform-soluble extract residue and analyze chromatographically as described in paragraph (d)(5)(iii)(b)(3)(i) of this section. In this case the weight of the dried residue from the heptane eluate must be multiplied by the dilution factor to obtain the weight of wax, petrolatum, and mineral oil residue to be subtracted from the weight of chloroform-soluble extractives residue (') to obtain the wax-, petrolatum-, and mineral oil-corrected chloroform-soluble extractives residue (e'). This e' is substituted for e in the equations in paragraph (d)(5)(i) (a) and (b) of this section. (Note: In the case of chloroform-soluble extracts which contain high melting waxes (melting point greater than **170** deg.F), it may be necessary to dilute the heptane solution further so that a 50-milliliter aliquot will contain only 0.1-0.2 gram of the chloroform-soluble extract residue.)

(e) Acrylonitrile copolymers identified in this section shall comply with the provisions of Sec. 180.22 of this chapter, except where the copolymers are restricted to use in contact with food only of the type identified in paragraph (c), table 1 under Category VIII.
[42 FR 14554, Mar. 15, 1977]

Editorial Note: For Federal Register citations affecting Sec. 176.**170**, see the List of CFR Sections Affected in the Finding Aids section of this volume.

6.3.F Substances for use only as components of adhesives in packaging which contacts food: PVP and PVPP

See following pages from Code of Federal Regulations.

000599 [redacted]

[Code of Federal Regulations]
[Title 21, Volume 3, Parts 170 to 199]
[Revised as of April 1, 1998]
From the U.S. Government Printing Office via GPO Access
[CITE: 21CFR175.105]

See pp. 15 and 17 (polymers of...)

[Page 138-153]

TITLE 21--FOOD AND DRUGS

CHAPTER I--FOOD AND DRUG ADMINISTRATION, DEPARTMENT OF HEALTH AND HUMAN SERVICES (

PART 175--INDIRECT FOOD ADDITIVES: ADHESIVES AND COMPONENTS OF COATINGS--Table of

Subpart B--Substances for Use Only as Components of Adhesives

Sec. 175.105 Adhesives.

(a) Adhesives may be safely used as components of articles intended for use in packaging, transporting, or holding food in accordance with the following prescribed conditions:

(1) The adhesive is prepared from one or more of the optional substances named in paragraph (c) of this section, subject to any prescribed limitations.

(2) The adhesive is either separated from the food by a functional barrier or used subject to the following additional limitations:

(i) In dry foods. The quantity of adhesive that contacts packaged dry food shall not exceed the limits of good manufacturing practice.

(ii) In fatty and aqueous foods. (a) The quantity of adhesive that contacts packaged fatty and aqueous foods shall not exceed the trace amount at seams and at the edge exposure between packaging laminates that may occur within the limits of good manufacturing practice.

(b) Under normal conditions of use the packaging seams or laminates will remain firmly bonded without visible separation.

(b) To assure safe usage of adhesives, the label of the finished adhesive container shall bear the statement ``food-packaging adhesive''.

(c) Subject to any limitation prescribed in this section and in any other regulation promulgated under section 409 of the Act which prescribes safe conditions of use for substances that may be employed as constituents of adhesives, the optional substances used in the formulation of adhesives may include the following:

(1) Substances generally recognized as safe for use in food or food packaging.

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(2) Substances permitted for use in adhesives by prior sanction or approval and employed under the specific conditions of use prescribed by such sanction or approval.

(3) Flavoring substances permitted for use in food by regulations in this part, provided that such flavoring substances are volatilized from the adhesives during the packaging fabrication process.

(4) Color additives approved for use in food.

(5) Substances permitted for use in adhesives by other regulations in this subchapter and substances named in this subparagraph: Provided, however, That any substance named in this paragraph and covered by a specific regulation in this subchapter, must meet any specifications in such regulation.

Substances	Limitations

000600

type), as the basic polymer, formed as esters when one or more of the following acids are made to react with one or more of the following alcohols:

Acids:

Azelaic acid.....

Dimethyl 1,4-cyclohexanedicarboxylate (CAS Reg. No. 94-60-0).

Dimethyl-5-sulfoisophthalic acid (CAS Reg. No. 50975-82-1) and/or its sodium salt (CAS Reg. No. 3965-55-7).

Polybasic and monobasic acids identified in Sec. 175.300(b)(3)(vii)(a) and (b).

5-sulfo-1,3-benzenedicarboxylic acid, monosodium salt (CAS Reg. No. 6362-79-4).

Tetrahydrophthalic acid.....

Alcohols:

1,4-Cyclohexanedimethanol.....

2,2-Dimethyl-1,3-propanediol...

1,6-Hexanediol (CAS Reg. No. 629-11-8).

Polyhydric and monohydric alcohols identified in Sec. 175.300(b)(3)(vii)(c) and (d).

Polyethyleneadipate modified with ethanolamine with the molar ratio of the amine to the adipic acid less than 0.1 to 1. — For use only in the preparation of polyurethan resins.

Polyethylene glycol (molecular weight 200-6,000).

Polyethyleneglycol alkyl(C<INF>10-C<INF>12) ether sulfosuccinate, disodium salt (CAS Reg. No. 68954-91-6)..

Polyethylene, oxidized.....

Polyethylene resins, carboxyl modified, identified in Sec. 177.1600 of this chapter.

Polyethylenimine.....

Polyethylenimine-epichlorohydrin resins

Polyisoprene.....

Polymeric esters of polyhydric alcohols and polycarboxylic acids prepared from glycerin and phthalic anhydride and modified with benzoic acid, castor oil, coconut oil, linseed oil, rosin, soybean oil, styrene, and vinyl toluene.

Polymers: Homopolymers and copolymers of the following monomers:.

Acrylamide.....

Acrylic acid.....

Acrylonitrile.....

Allylmethacrylate (CAS Reg. No. 00096-05-09).

Butadiene.....

Butene.....

N-tert-Butylacrylamide.....

Butyl acrylate.....

1,3-Butylene glycol dimethacrylate...

Butyl methacrylate.....

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Vinyl propionate.....
Vinyl pyrrolidone.....
Vinyl stearate.....
Polyoxyalkylated-phenolic resin (phenolic resin obtained from formaldehyde plus butyl- and/or amylphenols, oxyalkylated with ethylene oxide and/or propylene oxide).
Poly(oxycaproyl) diols and triols (minimum molecular weight 500).
Polyoxyethylated (40 moles) tallow alcohol sulfate, sodium salt.
Polyoxyethylene (20 mol)--anhydrous lanolin adduct.
Polyoxyethylene (molecular weight 200) dibenzoate.
Polyoxyethylene (molecular weight 200-600) esters of fatty acids derived from animal or vegetable fats and oils (including tall oil).
Polyoxyethylene (15 moles) ester of rosin.
Polyoxyethylene (4-5 moles) ether of phenol.
Polyoxyethylene (25 moles)--glycerol adduct.
Polyoxyethylene (40 moles) stearate....
Polyoxyethylene (5-15 moles) tridecyl alcohol.
Polyoxypropylene (3 moles) tridecyl alcohol sulfate.
Polyoxypropylene (20 moles) butyl ether
Polyoxypropylene (40 moles) butyl ether
Polyoxypropylene (20 moles) oleate butyl ether.
Polyoxypropylene-polyoxyethylene condensate (minimum molecular weight 1,900).
Polypropylene glycol (minimum molecular weight 150).
Polypropylene glycol (3-4 moles) triether with 2-ethyl-2-(hydroxymethyl)-1,3-propane-diol, average molecular weight 730.
Polypropylene glycol dibenzoate (CAS Reg. No. 72245-46-6). — For use as a plasticizer at levels not to exceed 20 percent by weight of the finished adhesive.

Polypropylene, noncrystalline.....
Polysiloxanes:
Diethyl polysiloxane.....
Dihydrogen polysiloxane.....
Dimethyl polysiloxane.....

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Diphenyl polysiloxane.....
Ethyl hydrogen polysiloxane.....
Ethyl phenyl polysiloxane.....
Methyl ethyl polysiloxane.....
Methyl hydrogen polysiloxane.....
Methyl phenyl polysiloxane.....
Phenyl hydrogen polysiloxane.....

000602

6.3.G Clarifying agent in beverages and vinegar: PVPP

See following pages from Code of Federal Regulations.

[Code of Federal Regulations]
[Title 21, Volume 3, Parts 170 to 199]
[Revised as of April 1, 1998]
From the U.S. Government Printing Office via GPO Access
[CITE: 21CFR173.50]

TITLE 21--FOOD AND DRUGS

CHAPTER I--FOOD AND DRUG ADMINISTRATION, DEPARTMENT OF HEALTH AND HUMAN SERVICES (CONTINUED)

PART 173--SECONDARY DIRECT FOOD ADDITIVES PERMITTED IN FOOD FOR HUMAN CONSUMPTION--Table of Contents

Subpart A--Polymer Substances and Polymer Adjuvants for Food Treatment

Sec. 173.50 **Polyvinylpolypyrrolidone.**

The food additive **polyvinylpolypyrrolidone** may be safely used in accordance with the following prescribed conditions:

(a) The additive is a homopolymer of purified vinylpyrrolidone catalytically produced under conditions producing polymerization and cross-linking such that an insoluble polymer is produced.

(b) The food additive is so processed that when the finished polymer is refluxed for 3 hours with water, 5 percent acetic acid, and 50 percent alcohol, no more than 50 parts per million of extractables is obtained with each solvent.

(c) It is used or intended for use as a clarifying agent in beverages and vinegar, followed by removal with filtration.

See page 116-117

[Code of Federal Regulations]
[Title 21, Volume 3, Parts 170 to 199]
[Revised as of April 1, 1998]
From the U.S. Government Printing Office via GPO Access
[CITE: 21CFR173]

[Page 110-137]

TITLE 21--FOOD AND DRUGS

CHAPTER I--FOOD AND DRUG ADMINISTRATION, DEPARTMENT OF HEALTH AND HUMAN SERVICES (CONTINUED)

PART 173--SECONDARY DIRECT FOOD ADDITIVES PERMITTED IN FOOD FOR HUMAN CONSUMPTION

Subpart A--Polymer Substances and Polymer Adjuvants for Food Treatment

Sec.
173.5 Acrylate-acrylamide resins.
173.10 Modified polyacrylamide resin.
173.20 Ion-exchange membranes.
173.21 Perfluorinated ion exchange membranes.
173.25 Ion-exchange resins.
173.40 Molecular sieve resins.
173.45 Polymaleic acid and its sodium salt.
173.50 **Polyvinylpolypyrrolidone.**
173.55 Polyvinylpyrrolidone.
173.60 Dimethylamine-epichlorohydrin copolymer.
173.65 Divinylbenzene copolymer.
173.70 Chloromethylated aminated styrene-divinylbenzene resin.
173.73 Sodium polyacrylate.
173.75 Sorbitan monooleate.

Subpart B--Enzyme Preparations and Microorganisms

173.110 Amyloglucosidase derived from Rhizopus niveus.
173.120 Carbohydrase and cellulase derived from Aspergillus niger.
173.130 Carbohydrase derived from Rhizopus oryzae.
173.135 Catalase derived from Microccocus lysodeikticus.
173.140 Esterase-lipase derived from Mucor miehei.
173.145 Alpha-Galactosidase derived from Mortierella vinaceae var. raffinoseutilizer.
173.150 Milk-clotting enzymes, microbial.
173.160 Candida guilliermondii.
173.165 Candida lipolytica.

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173.170 Aminoglycoside 3'-phosphotransferase II.

Subpart C--Solvents, Lubricants, Release Agents and Related Substances

173.210 Acetone.
173.220 1,3-Butylene glycol.
173.228 Ethyl acetate.
173.230 Ethylene dichloride.
173.240 Isopropyl alcohol.

(a) The molecular sieve resins consist of purified dextran having an average molecular weight of 40,000, cross-linked with epichlorohydrin in a ratio of 1 part of dextran to 10 parts of epichlorohydrin, to give a stable three dimensional structure. The resins have a pore size of 2.0 to 3.0 milliliters per gram of dry resin (expressed in terms of water regain), and a particle size of 10 to 300 microns.

(b) The molecular sieve resins are thoroughly washed with potable water prior to their first use in contact with food.

(c) Molecular sieve resins are used as the gel filtration media in the final purification of partially delactosed whey. The gel bed shall be maintained in a sanitary manner in accordance with good manufacturing practice so as to prevent microbial build-up on the bed and adulteration of the product.

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Sec. 173.45 Polymaleic acid and its sodium salt.

Polymaleic acid (CAS Reg. No. 26099-09-2) and its sodium salt (CAS Reg. No. 70247-90-4) may be safely used in food in accordance with the following prescribed conditions:

(a) The additives have a weight-average molecular weight in the range of 540 to 850 and a number-average molecular weight in the range of 520 to 650, calculated as the acid. Molecular weights shall be determined by a method entitled ``Determination of Molecular Weight Distribution of Poly(Maleic) Acid,'' March 17, 1992, produced by Ciba-Geigy, Inc., Seven Skyline Dr., Hawthorne, NY 10532-2188, which is incorporated by reference in accordance with 5 U.S.C. 552(a) and 1 CFR part 51. Copies are available from the Division of Product Policy, Center for Food Safety and Applied Nutrition (HFS-205), Food and Drug Administration, 200 C St. SW., Washington, DC 20204, or are available for inspection at the Center for Food Safety and Applied Nutrition's Library, 200 C St. SW., rm. 3321, Washington, DC, or at the Office of the Federal Register, 800 North Capitol St. NW., suite 700, Washington, DC.

(b) The additives may be used, individually or together, in the processing of beet sugar juice and liquor or of cane sugar juice and liquor to control mineral scale.

(c) The additives are to be used so that the amount of either or both additives does not exceed 4 parts per million (calculated as the acid) by weight of the beet or cane sugar juice or liquor process stream.

[51 FR 5315, Feb. 13, 1986, as amended at 61 FR 386, Jan. 5, 1996]

Sec. 173.50 **Polyvinylpolypyrrolidone.**

The food additive **polyvinylpolypyrrolidone** may be safely used in accordance with the following prescribed conditions:

(a) The additive is a homopolymer of purified vinylpyrrolidone catalytically produced under conditions producing polymerization and cross-linking such that an insoluble polymer is produced.

(b) The food additive is so processed that when the finished polymer is refluxed for 3 hours with water, 5 percent acetic acid, and 50 percent alcohol, no more than 50 parts per million of extractables is obtained with each solvent.

(c) It is used or intended for use as a clarifying agent in beverages and vinegar, followed by removal with filtration.

Sec. 173.55 Polyvinylpyrrolidone.

The food additive polyvinylpyrroli-done may be safely used in accordance with the following prescribed conditions:

(a) The additive is a polymer of purified vinylpyrrolidone catalytically produced, having an average molecular weight of 40,000 and a maximum unsaturation of 1 percent, calculated as the monomer, except that the polyvinylpyrrolidone used in beer is that having an average molecular weight of 360,000 and a maximum unsaturation of 1 percent, calculated as the monomer.

(b) The additive is used or intended for use in foods as follows:

Food	Limitations
Beer	As a clarifying agent, at a residual level not to exceed 10 parts per million.
Flavor concentrates in tablet form.	As a tableting adjuvent in an amount not to exceed good manufacturing practice.
Nonnutritive sweeteners in concentrated liquid form.	As a stabilizer, bodying agent, and dispersant, in an amount not to exceed good manufacturing practice.
Nonnutritive sweeteners in tablet form.	As a tableting adjuvant in an amount not to exceed good manufacturing practice.
Vitamin and mineral concentrates in liquid form.	As a stabilizer, bodying agent, and dispersant, in an amount not to exceed good manufacturing practice.
Vitamin and mineral concentrates in tablet form.	As a tableting adjuvant in an amount not to exceed good manufacturing practice.
Vinegar	As a clarifying agent, at a residual level not to exceed 40 parts per million.
Wine	As a clarifying agent, at a residual level not to exceed 60 parts per million.

According to ISP, PVPP is used at levels of 20-80mg per tablet

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Sec. 173.60 Dimethylamine-epichlorohydrin copolymer.

Dimethylamine-epichlorohydrin copolymer (CAS Reg. No. 25988-97-0) may be safely used in food in accordance with the following prescribed conditions:

(a) The food additive is produced by copolymerization of dimethylamine and epichlorohydrin in which not more than 5 mole-percent of dimethylamine may be replaced by an equimolar amount of ethylenediamine, and in which the mole ratio of total amine to epichlorohydrin is approximately 1:1.

(b) The additive meets the following specifications:

(1) The nitrogen content of the copolymer is 9.4 to 10.8 weight percent on a dry basis.

(2) A 50-percent-by-weight aqueous solution of the copolymer has a minimum viscosity of 175 centipoises at 25 deg.C as determined by LVT-series Brookfield viscometer using a No. 2 spindle at 60 RPM (or by another equivalent method).

(3) The additive contains not more than 1,000 parts per million of

6.3.H Clarifying agent in beverages and vinegar: PVP

See following pages from Code of Federal Regulations.

[Code of Federal Regulations]
[Title 21, Volume 3, Parts 170 to 199]
[Revised as of April 1, 1998]
From the U.S. Government Printing Office via GPO Access
[CITE: 21CFR173.55]

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TITLE 21--FOOD AND DRUGS

CHAPTER I--FOOD AND DRUG ADMINISTRATION, DEPARTMENT OF HEALTH AND HUMAN SERVICES (

PART 173--SECONDARY DIRECT FOOD ADDITIVES PERMITTED IN FOOD FOR HUMAN CONSUMPTION-

Subpart A--Polymer Substances and Polymer Adjuvants for Food Treatment

Sec. 173.55 Polyvinylpyrrolidone.

The food additive polyvinylpyrroli-done may be safely used in accordance with the following prescribed conditions:

(a) The additive is a polymer of purified vinylpyrrolidone catalytically produced, having an average molecular weight of 40,000 and a maximum unsaturation of 1 percent, calculated as the monomer, except that the polyvinylpyrrolidone used in beer is that having an average molecular weight of 360,000 and a maximum unsaturation of 1 percent, calculated as the monomer.

(b) The additive is used or intended for use in foods as follows:

Food	Limitations
Beer	As a clarifying agent, at a residual level not to exceed 10 parts per million.
Flavor concentrates in tablet form.	As a tableting adjuvent in an amount not to exceed good manufacturing practice.
Nonnutritive sweeteners in concentrated liquid form.	As a stabilizer, bodying agent, and dispersant, in an amount not to exceed good manufacturing practice.
Nonnutritive sweeteners in tablet form.	As a tableting adjuvant in an amount not to exceed good manufacturing practice.
Vitamin and mineral concentrates in liquid form.	As a stabilizer, bodying agent, and dispersant, in an amount not to exceed good manufacturing practice.
Vitamin and mineral concentrates in tablet form.	As a tableting adjuvant in an amount not to exceed good manufacturing practice.
Vinegar	As a clarifying agent, at a residual level not to exceed 40 parts per million.
Wine	As a clarifying agent, at a residual level not to exceed 60 parts per million.

000609

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6.4 ADI review of Crospovidone, WHO Report of 27th JECFA, 1983

The following pages have been extracted from the "Twenty-seventh Report of the Joint FAO/WHO Expert Committee on Food Additives", published by the World Health Organization as No. 696 in the Technical Report Series. These pages refer specifically to an evaluation of PVPP and PVP. The committee concluded that PVPP has the status "ADI not specified". This means that on the basis of available data (chemical, biological, toxicological, and other) the total daily intake of the substance, arising from its use at the levels necessary to achieve the desired effect and from its acceptable background in food, does not, in the opinion of the Committee, represent a hazard to health. For this reason and for the reasons stated in individual evaluations, the establishment of an acceptable daily intake (ADI) is not deemed necessary.

Pages 000611- 000621 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

6.5 Several Summaries of Original Works used in JECFA's review of PVPP

In the Agency Response Leter, GRAS Notice No. GRN 000030, the agency stated, " the generally available JECFA report is an opinion based on data and information that are not generally available…". We have been informed by ISP that the information submitted to the JECFA committee is not available because it is proprietary and handled in much the same way Drug Master Files are handled by the FDA. ISP has stated that much of the information is available in the Drug Master File, Type V #2297, held by the FDA. We do not have access to this file. However, your group should be able to obtain it since you are part of the same government agency.

The following pages concerning original work submitted to JECFA have been provided by Dr. James Griffiths of International Specialty Products (ISP) a subsidiary of GAF. ISP is the manufacturer of PVP and PVPP. Dr. Griffiths was kind enough to supply us with any non-proprietary information on PVPP that he had in his historical files. As stated in his introductory letter much of the information is already at the FDA in a Drug Master File.

International Specialty Products
1361 Alps Road Wayne, NJ 07470 • Tel: 973-628-4000

Facsimile Cover Sheet

To: Dr. Ursula Snow
Company: Shanstar Biotech Inc.
Phone: 510-594-1252
Fax: 507-242-0678

From: Dr. James C. Griffiths
Company: International Specialty Products
Phone: 973-628-4087
Fax: 973-628-4180

Date: 06/02/00
Pages with cover: ~~6~~ 8

Dear Dr. Snow,

As per previous conversations and your recent voice message let me respond as follows. In going through the GAF / ISP toxicological files I can only provide a few of the requested studies as the remainder were not developed nor paid for solely by GAF / ISP, and are part of the confidential submissions made to regulatory bodies worldwide, such as JECFA and the US FDA, food additive petitions and Drug Master File (DMF) by other manufacturers. The studies I can share are:

- 90-Day Feeding Study in rats, from January, 1976 000623
- DiGenis, Oral Absorption study of PVPP, Sept. 1979.
- Istin, Study of the urinary, biliary, and fecal execution of 14C by rats treated with labeled PVPP by gastric intubation, Sept. 1979.

[redacted]

[redacted]

I have attached the summary from each of these to this cover sheet. The acute LD50 work, as well as any human clinical studies are not in my files and reside at regulatory agencies for their review via confidential submissions and access.

What other data gaps can be addressed from my limited files? I am currently working on a proposal to have an independent GRAS expert panel deliberate the safety and exposure to PVPP in dietary supplements, via food uses currently paralleling pharmaceutical excipient uses.

I will update you with the scheduling and process as it becomes solidified.

Kind regards,

Jim(JCG458/00)

#7

FDRL

ABSTRACT

A ninety day feeding study has been completed in which rats have been fed diets containing 2% and 10% polyvinylpolypyrrolidone (PVPP), respectively. No changes were observed in food consumption, body weight gain, efficiency of food utilization or any biochemical or hematological parameter examined.

Complete gross pathological observations of all animals and microscopic observations of representative numbers of treated and control rats indicated that no adverse responses to dietary PVPP occurred in these rats.

Therefore it is concluded that PVPP at dietary concentrations of 2% and 10%, respectively, is well tolerated in FDRL/Wistar rats, and the no-adverse effect level of PVPP in this strain of rat is in excess of 10% of the total diet.

000625

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PRELIMINARY REPORT

Report 3 September 19, 1979

Oral Absorption Study Of PVPP

A study to determine the absorption, if any, of PVPP when administered by gastric gavage

by

George A. Digenis, Ph.D.

The objective of this study was to determine the amount of ^{14}C from ^{14}C-PVPP absorbed across the gastric or intestinal mucosa when administered orally to rats.

Summary Of Results

Less than one percent of the total ^{14}C dose from orally administered ^{14}C-PVPP was detected in the major organs, blood and urine samples during 6, 12, 24 and 48-hour observations. Feces collected during the 12, 24 and 48-hour intervals exhibit considerable ^{14}C-activity. The averages observed were 63.65% dose, 70.53% dose and 74.47% dose respectively. (Very little activity was detected in feces collected during the first six hours following administration of ^{14}C-PVPP.) In conclusion, PVPP seems relatively impermeable to the gastric and intestinal mucosas when administered orally to rats.

25-29, rue du Docteur Finlay

75015 Paris - Tel. 577.96.16

#2

STUDY OF THE URINARY, BILIARY, AND FECAL EXCRETION OF ^{14}C BY RATS TREATED WITH LABELED POLYVINYLPOLYPYRROLIDONE (PVPP-^{14}C) BY GASTRIC INTUBATION

[redacted]

[redacted]

SUMMARY AND CONCLUSIONS

A study on the absorption and excretion of carbon 14 was carried out in rats by the use of polyvinylpolypyrrolidone labeled with radiocarbon (PVPP-^{14}C).

The molecule was synthesized by the Laboratoires GAF. The labeled compound was administered to the animals in a suspension in distilled water by gastric intubation. Before it was used, the suspension was freed of labeled molecules with a molecular weight below 10,000 by ultrafiltration.

The most important results of this study were as follows:

- The absorbed fraction is small and represents only about 0.13% of the dose that was administered to the animals.

- The principal excretory route is through the kidneys, the bile accounting for only less than 6% of the total amount excreted.

- The kinetics of the urinary excretion are represented by a single exponential curve with a half-value time of 22 hours.

- None of the organs examined binds the ^{14}C-labeled compound specifically.

6.6 Review of clinical studies of PVP (Povidone), Lewis Publishers 1990

The following pages have been extracted from the monograph "PVP - A Critical Review of the Kinetics and Toxicology of Polyvinylpyrrolidone (Povidone)", edited b . V. Robinson, F. M. Sullivan, J. F. Borzelleca and S. L. Schwartz. These pages refer to an evaluation of PVP. We include this information here because PVP and PVPP are closely related and clinical studies are somewhat pertinent. Chemists opine that crosslinked PVP, or PVPP, is more inert than PVP. Thus results of any clinical studies should only show PVPP to be absorbed less by the body than PVP.

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[redacted]

[redacted]

Pages 000630- 000643 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

Reference List for Industry Submission, GRN 000030

Page Numbers	Publication
000611-000621	WHO, Evaluation of certain food additives and contaminants, 27th Report of the Joint FAO/WHO Expert Committee on Food Additives, WHO Technical Report Series 696, 1983, WHO, Geneva Switzerland.
000630-000643	Robinson, B.V.; Sullivan, F.M., Borzelleca, J.F.; Schwartz, S. L.; A Critical Review of the Kinetics and Toxicology of Polyvinylpyrolidone (Providone), 1990, Lewis Publishers