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August 22, 2006

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Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
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Phone: (202)-418-3090

REC'D AUG 24 2006

Dear Dr. Tarantino,

Please find enclosed three copies of the "Reduced-Pyruvate Xanthan Gum (RPXG) GRAS Notification". Please feel free to contact me with any questions or comments.

Sincerely.

J. David Luedeke, M.S.
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August 21, 2006

REC'D AUG 24 2006

Laura Tarantino, Ph.D.
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835

RE: Reduced-Pyruvate Xanthan Gum (RPXG) GRAS Notification

Dear Dr. Tarantino:

In accordance with proposed 21 CFR § 170.36 (a notice of a claim for exemption based on a GRAS determination) published in the Federal Register (62 FR 18937-18964), I am submitting in triplicate, as the representative of the notifier, CP Kelco, 8355 Aero Drive, San Diego, CA 92123, a GRAS notification for reduced-pyruvate xanthan gum (RPXG) for use as a food ingredient, such that the total daily consumption of RPXG would not exceed, and would partially replace, the current consumption of the GRAS ingredient, xanthan gum. A GRAS expert panel dossier, setting forth the basis for the GRAS determination, as well as CVs of the members of the GRAS panel, is enclosed.

Best regards,

George A. Burdock, Ph.D.
Diplomate, American Board of Toxicology
Fellow, American College of Nutrition

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1. GRAS Exemption Claim

A. Claim of Exemption from the Requirement for Premarket Approval Pursuant to Proposed 21 CFR 170.36 (c) (1)

Reduced-pyruvate xanthan gum (RPXG) has been determined to be generally recognized as safe (GRAS) and therefore, exempt from the requirement of premarket approval, under the conditions of its intended use as described below. The basis for this finding is described in the following sections.

Signed,

21 Aug 06

Date

George A. Burdock, Ph.D.
Diplomate, American Board of Toxicology
Fellow, American College of Nutrition
Burdock Group
888 Seventeenth Street, NW, Suite 810
Washington, DC 20006

(i) Name and Address of the Notifier

Mr. Rick Green, M.S.
Director, Regulatory Affairs
CP Kelco
8355 Aero Drive
San Diego, CA 92123

Agent of Notifier:

George A. Burdock, Ph.D.
Diplomate, American Board of Toxicology
Fellow, American College of Nutrition
Burdock Group
888 Seventeenth Street, NW, Suite 810
Washington, DC 20006

Telephone: 202-785-8200
Facsimile: 202-785-8666
Email: gburdock@burdockgroup.com

(ii) Common Name of the Notified Substance

The common name of reduced-pyruvate xanthan gum (RPXG) has been defined as:

Reduced-pyruvate xanthan gum (RPXG)

(iii) Conditions of Use

RPXG may be used as a stabilizer, emulsifier, thickener, suspending agent, bodying agent, or foam enhancer in foods, provided that food standards of identity do not preclude such use.[1] Application levels of RPXG are, in general, the same as for commercially available xanthan gum.

(iv) Basis of GRAS Determination

Pursuant to 21 CFR § 170.3, reduced-pyruvate xanthan gum (RPXG) has been determined GRAS by scientific procedures for its intended conditions of use. The safety of RPXG is supported by the fact that RPXG is substantially equivalent to xanthan gum, which has been

[1] Title 21 of the US Code of Federal Regulations (CFR), section 172.695, 2005

consumed as a food ingredient in the US for decades. This determination is based on the views of experts who are qualified by scientific training and experience to evaluate the safety of substances used as ingredients in food.

(v) Availability of Information

The data and information that serve as a basis for this GRAS determination are available for the Food and Drug Administration's (FDA) review and copying at a reasonable time at the office of:

George A. Burdock, Ph.D.
Diplomate, American Board of Toxicology
Fellow, American College of Nutrition
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Alternatively, copies of data and information can be provided to FDA upon request, by contacting Dr. Burdock.

2. Detailed Information About the Identity of the Notified Substance

A. Identity

Reduced-pyruvate xanthan gum (RPXG) is a high-molecular-weight extracellular polysaccharide gum produced by a pure-culture fermentation of a carbohydrate with the bacterium, *Xanthomonas campestris*, purified by recovery with isopropyl alcohol, and is composed of β-D-glucosyl, α-D-mannosyl, β-D-mannosyl, and β-D-glucosyluronic acid residues and differing proportions of acetyl and pyruvate groups. The primary structure consists of a cellulose backbone with trisaccharide side chains, and with a pentasaccharide as a repeating unit. RPXG is made directly from a fermentation process utilizing *X. campestris* in a medium containing a fermentable sugar, *e.g.*, glucose, sucrose or corn syrup, with organic and inorganic nitrogen sources. RPXG is dried, milled and is readily soluble in hot or cold water, obtaining a neutral solution. RPXG is produced under current Good Manufacturing Practices (cGMP) as a food grade ingredient, for use as a stabilizer, emulsifier, thickener, suspending agent, bodying agent, or foam enhancer in foods for which standards of identity do not preclude such use (Table 1).

Table 1. General description and analytical information of reduced-pyruvate xanthan gum (RPXG)

Bacterial Source	*Xanthomonas campestris*
Physical description	Cream-colored powder
Packaging	Packaged in laminate bags.
Storage	Store at 25°C.
Labeling	Reduced-pyruvate xanthan gum.
Functional Use in Food	A stabilizer, emulsifier, thickener, suspending agent, bodying agent, or foam enhancer in foods for which standards of identity do not preclude such use.

The content of pyruvate in gums has previously been utilized as an identification of xanthan gum.[2] Xanthan gum was found to be one of the few gums that, on average, contained substantial (*e.g.*, greater than 1.5%) levels of pyruvate. As RPXG produced under typical fermentation conditions conforms to all manufacturing specifications as commercial xanthan gum, except the regulatory standards for pyruvate content, other methods must be established to uniquely identify RPXG, when compared with other types of gums. Table 2 indicates that use of the brine solubility test, the pyruvic acid test, and the locust bean gum gel test, may be utilized to uniquely characterize RPXG, when compared with other types of gum substances.[3]

Table 2. Unique characteristics that identify reduced-pyruvate xanthan gum (RPXG) from other gums

Food Additive (lot #)	Brine Solubility	Pyruvic Acid (%)	Locust Bean Gum Gel Test
Xanthan gum (514287A)	Fail	4.55 %	Pass
Reduced-pyruvate xanthan gum (05-686)	Pass	0.22 %	Pass
Gellan gum (4K1465)	Fail	0.21 %	Fail
CMC (NSP42112)	Fail	0.16 %	Fail
Pectin (S53003)	Fail	1.07 %	Fail
Carrageenan (S52554)	Fail	1.83 %	Fail

CMC = Sodium carboxymethyl cellulose gum

Common or Usual Name:

The common name of reduced-pyruvate xanthan gum has been defined as:

Reduced-pyruvate xanthan gum

[2] Food Chemicals Codex
[3] Test protocols available upon request

B. Composition

The chemical characteristics of RPXG are summarized in Table 3. RPXG is a water-soluble polysaccharide gum produced from *Xanthomonas campestris*, and is composed of β-D-glucosyl, α-D-mannosyl, β-D-mannosyl, and β-D-glucosyluronic acid residues and differing proportions of acetyl and pyruvate groups. The primary structure consists of a cellulose backbone with trisaccharide side chains, and with a pentasaccharide as a repeating unit. RPXG contains a reduced concentration of the pyruvate moiety. The method of manufacture of RPXG is indicated in Figure 1.

Table 3. Chemical characteristics of reduced-pyruvate xanthan gum (RPXG)

Characteristic	Value
Appearance	Cream-colored powder
Assay*	A sample yields, on the dried basis, not less than 4.2% and not more than 5.4% of carbon dioxide (CO_2), corresponding to between 91.0% and 117.0% of Xanthan Gum
Molecular weight	Approximately 2,000 Kilodaltons
Solubility	Readily dissolves in water with stirring to give highly viscous solutions at low concentrations
Viscosity	Passes Food Chemicals Codex test

*Concurrent with the Food Chemicals Codex chemical characteristics of xanthan gum, except the reduction in the pyruvic acid content

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C. Method of Manufacture of Reduced-Pyruvate Xanthan Gum

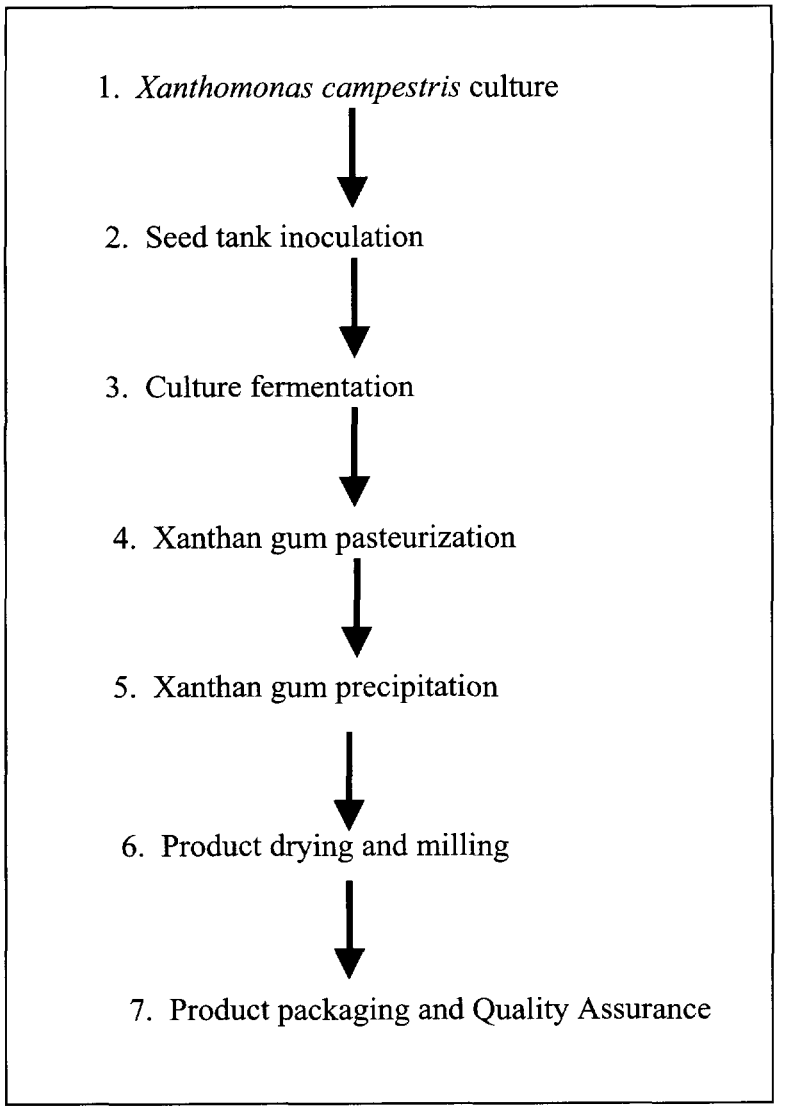

Figure 1. Reduced-pyruvate xanthan gum (RPXG) production scheme

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D. Specifications for Food Grade RPXG

Table 4. Specifications of reduced-pyruvate xanthan gum (RPXG)

Identification tests	
Ash (%)	Not more than 16.0
Assay* (%)	91.0-117.0, dried basis
Locust bean gum gel test	Positive/Pass
1% Potassium chloride viscosity (cP)	Not less than 600
Pyruvic acid (%)	<1.5
Viscosity ratio	1.02-1.45
Arsenic (ppm)	<3
Carbon dioxide (%)	4.2 to 5.4
Heavy metals (ppm)	Not more than 20
Isopropyl alcohol (ppm)	Not more than 750
Lead (ppm)	<2.0
Loss on drying (%)	Not more than 15.0

cP = centipoise; ppm = parts *per* million; *Food Chemicals Codex assay, in which a sample yields, on the dried basis, not less than 4.2% and not more than 5.4% of carbon dioxide (CO_2), corresponding to between 91.0 and 117.0% of xanthan gum

3. Self Limiting Levels of Use

At high levels, certain food items may acquire an undesirably viscous nature.

4. Basis of GRAS Determination

The determination that reduced-pyruvate xanthan gum is GRAS is on the basis of scientific procedures. See attached- DOSSIER IN SUPPORT OF THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS REDUCED-PYRUVATE XANTHAN GUM (RPXG) AS A FOOD INGREDIENT. On the basis of the data and information described in the attached dossier and other publicly available information, there is consensus, among experts qualified by scientific training and experience to evaluate the safety of substances added to food, that there is reasonable certainty that reduced-pyruvate xanthan gum is generally recognized as safe under the intended conditions of use.

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FINAL

DOSSIER IN SUPPORT OF THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF REDUCED-PYRUVATE XANTHAN GUM (RPXG) AS A FOOD INGREDIENT

June 14, 2006

Panel Members

George A. Burdock, Ph.D.

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DOSSIER IN SUPPORT OF THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF REDUCED-PYRUVATE XANTHAN GUM (RPXG) AS A FOOD INGREDIENT

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FINAL

DOSSIER IN SUPPORT OF THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF REDUCED-PYRUVATE XANTHAN GUM (RPXG) AS A FOOD INGREDIENT

1. EXECUTIVE SUMMARY

The undersigned, an independent panel of recognized experts (hereinafter referred to as the Expert Panel),[1] qualified by their scientific training and relevant national and international experience to evaluate the safety of food ingredients, was requested by CP Kelco to determine the Generally Recognized As Safe (GRAS) status, based on scientific procedures, of reduced-pyruvate xanthan gum (RPXG). RPXG is to be used as an ingredient to function as a stabilizer, emulsifier, thickener, suspending and bodying agent, and foam enhancer, replacing the use of commercially available xanthan gum in certain applications, at an average consumption of 14.73 mg/kg/day. In particular, the Expert Panel has evaluated the data concerning xanthan gum and RPXG, and has determined substantial equivalency between xanthan gum and RPXG. A comprehensive search of the scientific literature was conducted for safety and toxicity information on xanthan gum and related compounds and, along with supporting documentation, was made available to the Expert Panel. In addition, the Expert Panel independently evaluated materials submitted by CP Kelco and other materials deemed appropriate and necessary. Following an independent, critical evaluation, the Expert Panel conferred and unanimously agreed to the decision described herein.

2. INTRODUCTION

Xanthan gum is a high-molecular-weight extracellular polysaccharide gum produced by a pure-culture fermentation of a carbohydrate with the bacterium, *Xanthomonas campestris*, purified by recovery with isopropyl alcohol. The matrix is then dried and milled, resulting in a cream-colored powder. Xanthan gum is utilized in numerous food and industrial applications for its ability to thicken or stabilize aqueous systems. Xanthan gum was approved as a food additive in 1969 by the United States Food and Drug Administration (US FDA), for use in accordance with current good manufacturing practice as a stabilizer, emulsifier, thickener, suspending agent, bodying agent, or foam enhancer in foods for which standards of identity established under Section 401 of the Act do not preclude such use.[2] Xanthan gum also received approval from the European Union under E-number 415 in 1980 (Becker *et al.*, 1998).

The primary structure of xanthan gum consists of repeating pentasaccharide units of two D-glucopyranosyl residues, one α-D-mannopyranosyl residue, one β-D-glucopyranosyluronic acid residue, and one terminal β-D-mannopyranosyl group (Jansson *et al.*, 1975; Melton *et al.*, 1976). The polymer backbone, made up of (1→4)-linked β-D-glucopyranosyl residues, is identical to that of cellulose. A trisaccharide side chain containing a (1→3)-linked α-D-mannopyranosyl residue, a (1→2)-linked β-D-glucopyranosyluronic acid, and a (1→4)-linked β-D-mannopyranosyl group is attached to alternating D-glucosyl residues of the polysaccharide backbone *via* an O-3 linkage. Non-carbohydrate substituents, including acetic acid and pyruvic acid, are found on xanthan gum as 6-O-acetyl esters on the inner mannose residue and on the

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[1] Modeled after that described in Section 201(s) of the Federal Food, Drug, and Cosmetic Act, as amended. See also attachments (*curriculum vitae*) documenting the expertise of the Panel members.
[2] Title 21 of the US Code of Federal Regulations (CFR), section 172.695, 2004

terminal mannose group, and 4,6-O-(1-carboxyethylidine) cyclic acetal on the terminal mannose group (Sloneker and Orentas, 1962). The pyruvic acid acetal is formed on the mannose unit at the non-reducing end of the pentasaccharide, first assembled on a pyrophosphate lipid and then polymerized to xanthan gum once the pentasaccharide is assembled (Ielpi *et al.*, 1981). Acetyl groups contained within the helix stabilize the ordered helix, while pyruvate groups destabilize the helix. A structural representation of xanthan gum is shown in Figure 1 (Hassler and Doherty, 1990; FCC, 2003). In addition to the repeating unit shown for RPXG, there are three other possibilities. (1) There may be no acetate groups at all, (2) there may be a second acetate group linked to the 6 position of the terminal mannose (through the CH_2OH group) or (3) there may be a single acetate group on the terminal mannose and not on the inner mannose sugar as shown. The amount of pyruvate found in commercial xanthan gum may vary, depending on the bacterial growth conditions. Decreased pyruvate concentrations in xanthan gum alter the pseudoplastic characteristics of RPXG; RPXG is more free flowing (*i.e.*, "pourable"), but more viscous when stationary. This is a highly desirable characteristic in liquid food systems. A form of xanthan gum has been produced by variant forms of *X. campestris* bacteria, as exemplified by RPXG produced by *X. campestris* ATCC 31313, that contains a reduced concentration of pyruvate; this dossier is a summary of the scientific evidence that supports the general recognition that the reduced-pyruvate xanthan gum is safe for human consumption as a food ingredient.

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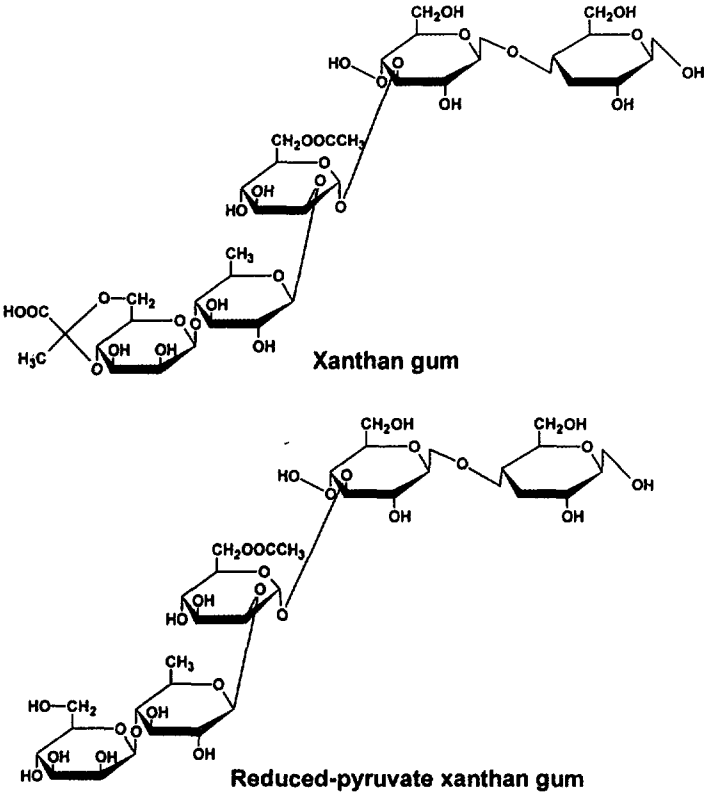

Figure 1. Structure of xanthan gum and the theoretical structure of reduced-pyruvate xanthan gum (RPXG) (Hassler and Doherty, 1990; FCC, 2003)

2.1. Composition of the Reduced-Pyruvate Xanthan Gum

The primary structure of reduced-pyruvate xanthan gum is identical to that of xanthan gum, with the exception of a reduced percentage of pyruvic acid groups contained in the polysaccharide molecule. Reduced-pyruvate xanthan gum, like the native polysaccharide, contains acetyl groups linked to the 6-position of the terminal mannose group (Stankowski *et al.*, 1993). Reduced-pyruvate xanthan gum utilizes nearly identical production methods, and contains the same constituents as standard xanthan gum, although having a reduced percentage of pyruvic acid.

Several techniques were used to demonstrate that reduced-pyruvate xanthan gum, produced from nonpathogenic/nontoxigenic *X. campestris* strains of bacteria typical of those strains used in the production of xanthan gum, contains less than 1.5% pyruvate. The techniques included: (1) IR[3] analysis, whereby absence of pyruvate moieties was demonstrated; and (2) HPLC[4] analysis, wherein approximately 0.5% of pyruvate was detected from xanthan gum

[3] Infrared analysis
[4] High-Performance Liquid Chromatography

produced from the variant *X. campestris* strain ATCC 31313 (grown under identical conditions to commercially produced xanthan gum), in comparison to about 5.5% from that made by the current production strain *X. campestris* XWCM-1, when cultured in typical xanthan gum production conditions. The reduced-pyruvate xanthan gum was also functionally less synergistic with guar gum (a galactomannan), a trait expected for a xanthan gum with reduced pyruvate content, and utilized as indirect evidence, which established that xanthan gum made from the example strain ATCC 31313 had significantly less pyruvate. Molecular weight studies indicate that xanthan gum is approximately 2,000 kilodaltons in size (Kelco Company, 1975), and the removal of the pyruvate moiety should not significantly alter the molecular weight of reduced-pyruvate xanthan gum.

Hassler and Doherty (1990) studied the effects of changes in the acetylation and/or pyruvylation of the repeating unit of xanthan gum on polymer solution viscosity properties. *X. campestris* mutants were cultured to produce samples of polymers that contained various levels of acetyl or pyruvate molecules. The *X. campestris* variants were cultured to produce polymer samples formulated to 0.05, 0.1, 0.15, and 0.2 *percent* weight in 0.1 M sodium chloride. Viscosities of the samples were measured at a constant shear rate at 25°C. The molar ratios of acetate and pyruvate *per* glucose of these variant polysaccharides, as well as their relative viscosities, are noted in Table 1. All non-pyruvylated xanthan variants were of significantly lower viscosity than their pyruvated counterparts. The highest viscosity was associated with the polymer that is pyruvated on the outer mannose (X1402), while the lowest viscosity was associated with the polymer that is acetylated on the outer mannose (X1401), while the viscosity of the polymer with an unmodified outer mannose (X1403) was of intermediate viscosity. This data indicates that pyruvylation of the outer mannose directly enhances viscosity approximately two-fold, as well as indirectly enhancing viscosity by blocking acetylation of the mannose residue. In summary, the presence of pyruvate increases viscosity, whereas acetate decreases viscosity.

Table 1. Viscosity results of *X. campestris* variants defective in acetylation and/or pyruvylation (Hassler and Doherty, 1990)

Molar ratios of substituents *per* glucose		Viscosity,[a] cP				Strain
Pyruvate	**Acetate**	**0.05 wt %**	**0.10 wt %**	**0.15 wt %**	**0.20 wt %**	
0.35	0.00	7.0	22.5	49.5	91.5	X1402
0.34	0.61	2.5	7.5	13.0	26.0	X1398
0.33	0.57	2.5	7.5	13.0	23.0	X1396
0.32	0.09	4.5	12.0	16.5[b]	NR	X1400
0.00	1.15	1.0	2.0	2.5	4.5	X1397
0.00	0.47	1.5	3.5	8.0	13.5	X1399
0.00	0.44	2.0	4.0	7.0	12.0	X1401
0.00	0.00	2.5	10.0	22.0	41.5	X1403

[a]Measured in 0.1 M NaCl at 25°C and $8s^{-1}$,using a Brookfield LVT viscometer. [b]0.12 wt %; NR=Not Reported

Under conditions of continuous fermentation of *X. campestris*, development of variant polysaccharide forms and diminished polysaccharide production have been observed (Silman and Rogovin, 1972). Variant colonies develop during maintenance of the microbial culture under propagative conditions, resulting in polysaccharides with variable aspects of composition and rheological properties. Different growth phases and alterations of the growth medium (*e.g.*,

limiting nutrients or the use of different substrates) do not influence the primary carbohydrate structure, but do affect the amount of pyruvylation, yield and molecular mass (Tait *et al.*, 1986; Becker *et al.*, 1998). Cadmus *et al.* (1976) noted that slight variations in culture conditions and sugar concentrations resulted in the production of both large- (PS-L) and small- (PS-Sm) colonies of *X. campestris*. In addition, when the characteristics of the polysaccharides from these strains were compared, it was reported that the rheological properties of PS-Sm resemble those of PS-L and the initial parent strain (PS-P). However, the pyruvic acid content of the polysaccharide formed from the PS-Sm colonies was approximately one-half the value of the PS-L and PS-P colonies (pyruvic acid content was 4.4 and 4.7 g/100g polysaccharide produced by PS-L and PS-P colonies, respectively, compared with 2.5 g/100g polysaccharide by PS-Sm colonies). Chemical analyses of the PS-L and PS-Sm polysaccharides found that both types of polysaccharides have identical ratios of component sugars, indicating that the basic structures were the same. These studies indicate that the basic structure of xanthan gum does not change when the concentration of pyruvic acid is altered.

As indicated in a Canadian patent (Jarman and Pace, 1985), cultivation of commonly used *X. campestris* production strains under certain media nutrient conditions, and in continuous culture, may result in the production of a xanthan gum that has a pyruvate content less than 1.5% and, in some instances, a xanthan gum with a pyruvate content near 0.1%.

Sandford *et al.* (1978) separated xanthan gum according to its differing pyruvate content *via* fractional precipitation with alcohol. The gum produced by the type strain *Xanthomonas campestris* NRRL B-1459[5] was ethanol fractionated, according to the amount of ethanol required for precipitation (*i.e.*, size of fraction *vs.* ethanol (%) required for its precipitation). Pyruvic acid was then measured colorimetrically. Results indicated that at least four different fractions were formed, with the lowest level reported at 1.0%, and the highest at 6.5% pyruvic acid (Table 2). This study indicates that the final xanthan gum product from the type strain of xanthan gum-producing bacteria may actually be a mixture of high- and low-pyruvate-containing gums.

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[5] Commercial xanthan gum produced by CP Kelco, CA.

Table 2. Fractionation of PS B-1459. Final products of differing pyruvic acid content (Sandford *et al.*, 1978)

Analysis of fractions						
				Main fraction		
Sample	Pyruvate content of parent material (%)	Highest pyruvate value (%)	Lowest pyruvate value (%)	Pyruvate content (%)	Ethanol required for precipitation[a] (%)	Size of fraction[b] (%)
A	1.3	1.6	1.0	1.0	38	15
B	3.2	4.2	2.4	3.4	44	13
C	4.9	5.9	4.2	5.9	47	31
D	5.9	6.5	4.0	6.5	49	73

[a]1% of KCl present; [b]percentage of unfractionated material

A xanthan polysaccharide, produced by *X. campestris* pv. *oryzae*, contains the same carbohydrate backbone, but with a lower percentage of pyruvic acid than commercially-produced xanthan gum (Table 3), and indicates that pyruvic acid may be present in non-stoichiometrical proportions in xanthan polysaccharides (Jansson *et al.*, 1975; Shatwell *et al.*, 1990). This alteration of the number of pyruvic acid groups may also be due to variations in the composition of the fermentation medium for *X. campestris*, as the production alterations that are needed when switching from batch fermentation to fed-batch fermentation procedures, as well as the addition of citric acid to the fermentation medium, alters the degree of acetylation and pyruvylation of the mannose in the xanthan gum molecule (Jana and Ghosh, 1999; Illiev and Ivanova, 2002).

Table 3. Microbial origin, chemical composition of polymers[a] and optical rotation midpoint temperatures[b] (Shatwell *et al.*, 1990)

Polymer	Bacterial strain	% Glucose	% Mannose	% Glucuronic acid	% Acetate	% Pyruvate	Tm (°C)
ps.646	*X. campestris* pv. *campestris* 646	40.2	36.0	23.8	4.5	4.4	44
ps.1128	*X. campestris* pv. *phaseoli* 1128	40.3	36.7	23.0	7.7	1.7	54.5
ps.PXO_{61}	*X. campestris* pv. *oryzae* PXO_{61}	40.6	37.7	21.7	14.3	0.3	49.5
ps.556	*X. campestris* pv. *phaseoli* 556	40.5	38.7	20.8	1.6	6.0	38

[a]The quantities of monosaccharide were expressed as a percentage of the total sugar content, while the amounts of acetyl and pyruvate were expressed as a percentage of the total weight including the acetyl content. [b]Measured in deionized water. Tm = the optical rotation midpoint temperatures; a determination of chain flexibility

Davidson (1978) demonstrated that limitations in phosphate or magnesium concentrations resulted in xanthan gum containing low levels of pyruvate (0.9% and 1.1% pyruvate, respectively). There was minimal effect on mannose: glucose ratios, *percent* glucuronic acid, or *percent* acetate parameters. Minor microenvironment fluctuations in mineral content in the xanthan gum production batch may alter the percentage of pyruvate contained in xanthan gum produced in batch processing. Studies evaluating lyases acting on xanthan gum

have isolated lyases from various bacterial species, such as *Bacillus* and *Corynebacterium* species (Sutherland, 1987). The action of the xanthan gum lyases derived from either *Bacillus* or *Corynebacterium* species was unaffected by the presence or absence of a 4,6-pyruvate ketal group on the terminal β-mannosyl side-chain residue of xanthan, indicating that the lyase can function in the presence of varying levels of pyruvate attached to the side chain, promoting degradation of xanthan gum in the environment.

2.2. Regulatory Status and Substantial Equivalence

US Food and Drug Administration's Center for Drug Evaluation and Research had approved the use of xanthan gum as a food ingredient, as well as an inactive ingredient in oral and topical pharmaceutical formulations (CDER, 2004). FDA regulatory specifications for xanthan gum (21 CFR Sec. 172.695 (d)(4) Xanthan gum) are presented in APPENDIX I, and include the assays for quantification of pyruvate.

An adequate safety assessment of novel food ingredients is important to regulatory agencies and the public. To address this issue, and yet avoid unnecessary and costly toxicology testing, agencies have developed the concept of substantial equivalence as a guide for food developers. In 1992, the FDA published a statement of policy on foods derived from new plant varieties, which may be applicable to bacterial strains (FDA, 1992). The scientific concepts described in that document are consistent with the concepts of substantial equivalence of new foods discussed in Safety of Genetically Engineered Foods, Approaches to Assessing Unintended Health Effects published by the Institute of Medicine (IoM and NRC, 2004), Strategies for Assessing the Safety of Foods Produced by Biotechnology, published by the World Health Organization (JECFA, 1991), Concepts and Principles for Safety Evaluation of Foods Derived by Modern Technology, published by the Organization of Economic Cooperation and Development (OECD, 1993), and Guidelines for the Safety Assessment of Novel Foods, published by Health Canada's Health Protection Branch Food Directorate (Health Canada, 1994). The OECD document concluded that if a new food is determined to be substantially equivalent, there is an expectation that the new food will be treated as if it was the conventional counterpart and any further safety or nutritional concerns are considered as insignificant and unexpected (OECD, 1993).

In its statement of policy, FDA (1992) cautioned against inadequate safety assessment for host plants with little or no history of safe use, but it also stated that the modification of host plants with a history of safe use would minimize adverse public health consequences and provide a basis for determining whether new plant varieties are as safe and nutritious as their parental varieties. Included among the information needed to establish substantial equivalence for foods with a history of safe use are consideration of (1) changes in known toxicants, (2) nutrient alternatives, (3) effects on allergenicity, and (4) introduction of unintended (*e.g.*, pleiotropic) effects into the host plant. This concept may be applied to food ingredients derived from variant bacterial strains isolated from bacterial strains with a history of safe use in the production of food ingredients.

Although the substantial equivalence concept was originally developed as an aid in assessing the safety of genetically modified foods, the OECD has advocated its application as the most practical approach to address the safety of foods and food components derived through traditional plant breeding technology (OECD, 1993). This concept can be extended to include

mutations in bacterial organisms that produce food components, such as the development of CP Kelco's reduced-pyruvate xanthan gum (RPXG). As previously indicated, RPXG meets all of the specifications of conventional xanthan gum, with the exception of a reduction in the pyruvate content. This is strong evidence that RPXG is similar to conventional xanthan gum and meets the intent of substantial equivalence as established by the above agencies.

FDA's "Statement of Policy: Foods Derived from New Plant Varieties" (FDA, 1992) contains several flow diagrams that illustrate steps to be taken to determine food safety from new plant varieties. This statement includes a proposed mechanism for the safety assessment of new or modified carbohydrates.[6] The flow chart provided in the statement (Figure 2) outlines three general concepts, with the questions and answers pertinent for the RPXG below:

1. "Has there been an intentional alteration in the structure, composition, or level of carbohydrates in the new variety?" The response is confirmatory, in that the intention is to produce *de novo* a reduced-pyruvate xanthan gum, a carbohydrate polysaccharide.

2. "Have any structural features or functional groups been introduced into the carbohydrate that do not normally occur in food carbohydrates?" No, reduced-pyruvate xanthan gum has been found as a normal variant in the production of xanthan gum from *X. campestris* bacteria containing the carbohydrate structure equivalent to currently produced xanthan gum (Davidson, 1978; Tait *et al.*, 1986; Becker *et al.*, 1998).

3. "Have there been any alterations that could affect digestibility or nutritional qualities in a carbohydrate that is likely to be a macroconstituent in the diet?" The reduction in pyruvate is not expected to alter the digestion of xanthan gum. Xanthan gum has been reported to pass through the digestive system relatively unchanged (Kelco Company, 1975; Eastwood *et al.*, 1987). The absence of pyruvate on the molecule would not affect the lack of digestibility of the xanthan gum.

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[6] Statement of Policy: Foods Derived from New Plant Varieties. "Safety Assessment of New Varieties: New or Modified Carbohydrates" (FDA, 1992).

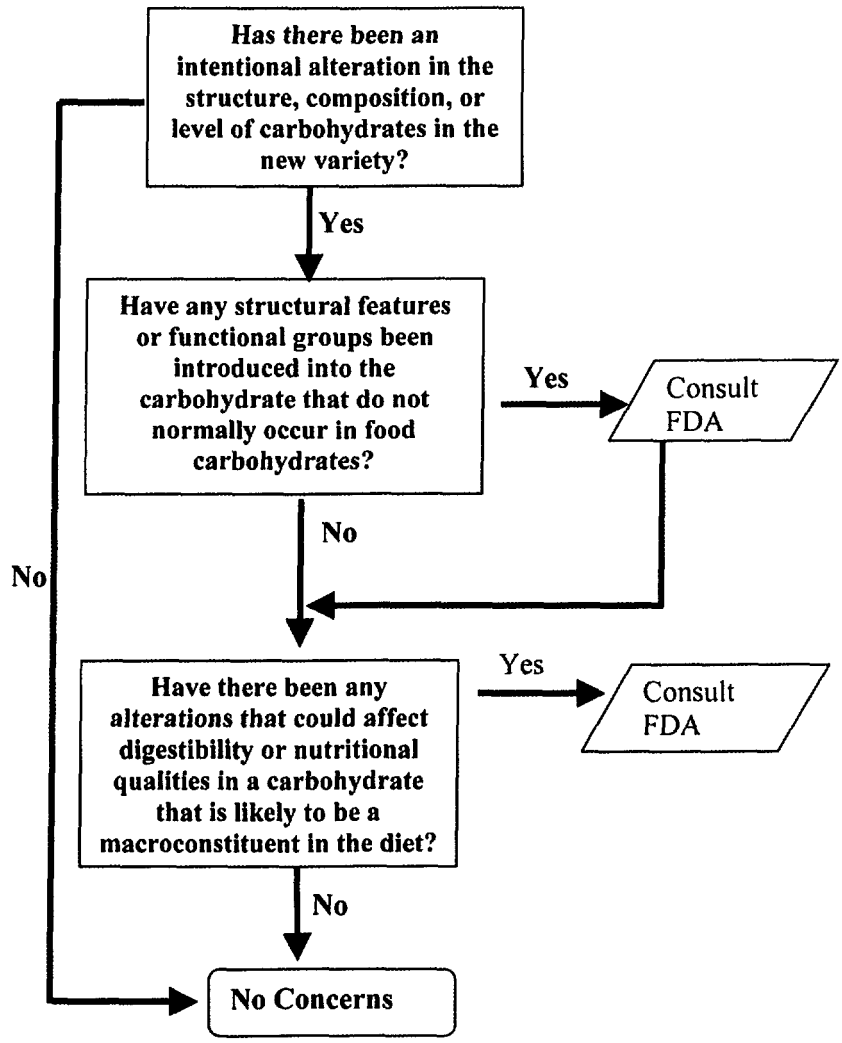

Figure 2. FDA Statement of Policy: Foods Derived From New Plant Varieties (FDA, 1992)

The outcome of this combination of questions and answers yields a "No concerns" finding for reduced-pyruvate xanthan gum (FDA, 1992). The reduced concentration of pyruvate typically found in end-production xanthan gum is only a minor variation in the chemical structure of xanthan gum (a removal, not an addition, of a chemical group), which does not affect the safety of xanthan gum, based on FDA's safety assessment paradigm for foods derived from new plant varieties. The xanthan gum contains an alteration that could normally occur as the result of variations in the growth patterns of variants of *X. campestris* bacteria, and variations in the growth conditions of fermenting *X. campestris*. As a result, there would be no effect on the

digestibility or nutritional quality (if any) of the xanthan gum (Figure 2). Xanthan gum is not a macroconstituent of the diet, nor does it add nutritive value to the diet.

In addition, the FDA flow chart of the "Safety Assessment of New Varieties: Summary" in FDA's "Statement of Policy: Foods Derived From New Plant Varieties"(FDA, 1992), as indicated in Figure 3, poses two questions under the Safety Assessment of New or modified carbohydrates, fats or oils, to which the answers are as follows:

1. "Are there any unusual or toxic components?" No; there are no toxic components associated with reduced-pyruvate xanthan gum.

2. "Are there any alterations that could affect nutritional qualities or digestibility in a macroconstituent of the diet? No; xanthan gum, in general, and reduced-pyruvate xanthan gum, specifically, is not digested in the human gastrointestinal tract, and is therefore not a macroconstituent of the diet.

A "No" answer to both of these questions yields an assessment of "No concerns," as established by the FDA (1992) (Figure 2). This information indicates that an alteration in the final bacterial fermentation product resulting in a reduced level of pyruvate, does not pose a concern for the use of reduced-pyruvate xanthan gum.

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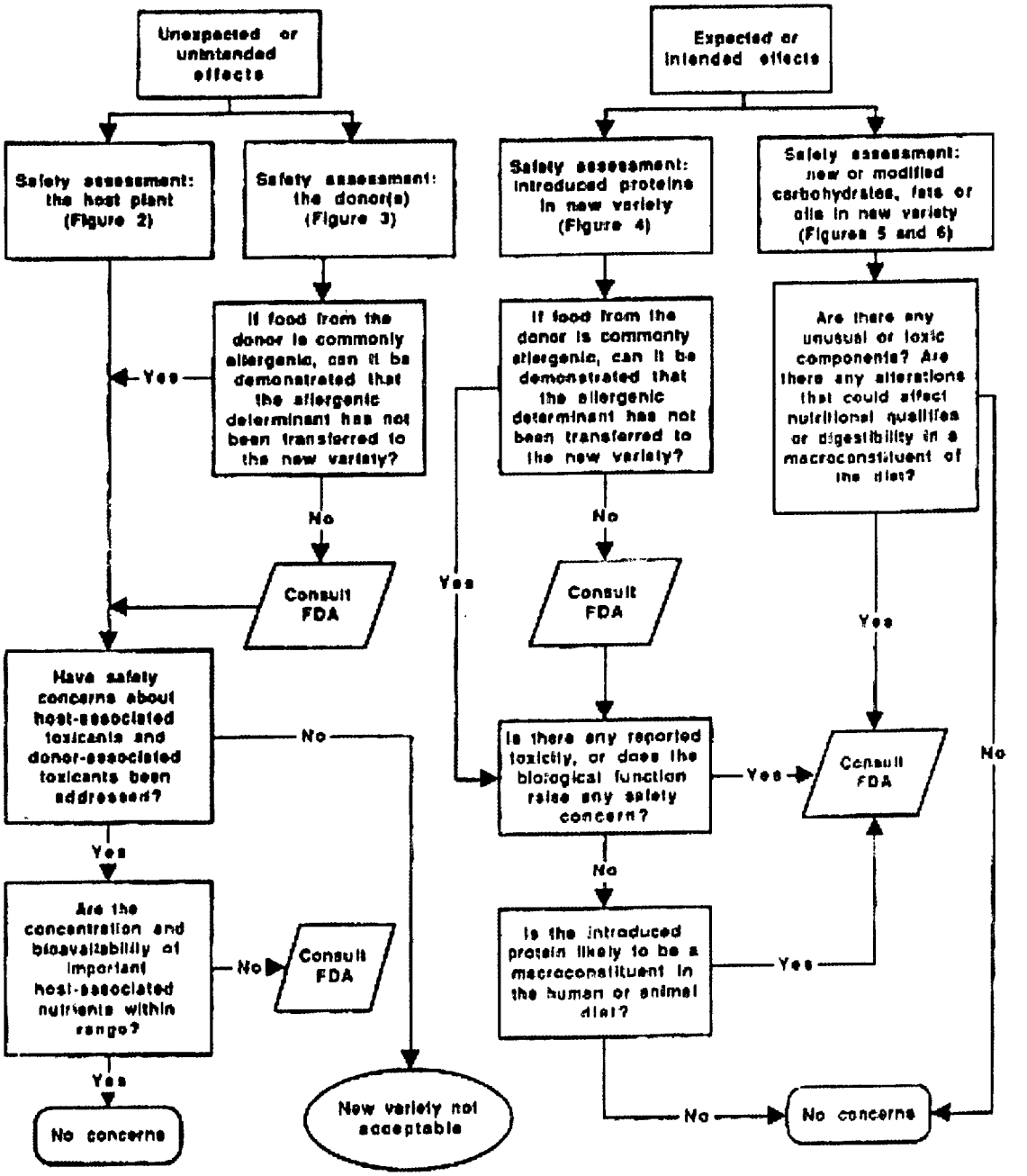

Figure 3. Flow chart describing the safety assessment of new carbohydrate varieties (FDA, 1992)

3. DESCRIPTION, MANUFACTURING PROCESS AND SPECIFICATIONS

3.1. Description

The physical and chemical properties of reduced-pyruvate xanthan gum (RPXG) and specifications for production of RPXG are provided in Table 4 and Table 5, respectively. RPXG is a polysaccharide gum produced by the bacterium *Xanthomonas campestris*, and is composed of β-D-glucosyl, α-D-mannosyl, β-D-mannosyl, and β-D-glucosyluronic acid residues and differing proportions of acetyl and pyruvate groups. The primary structure consists of a cellulose backbone with trisaccharide side chains, and with a pentasaccharide as a repeating unit (Sloneker and Orentas, 1962; Jansson *et al.*, 1975). Reduced-pyruvate xanthan gum is made directly from fermentation process utilizing *X. campestris* in a medium containing a fermentable sugar, *e.g.*, glucose, sucrose or corn syrup, with organic and inorganic nitrogen sources.

RPXG is dried, milled and is readily soluble in hot or cold water, obtaining a neutral solution. RPXG may be used as a stabilizer, emulsifier, thickener, suspending agent, bodying agent, or foam enhancer in foods, provided that food standards of identity do not preclude such use (FDA, 2005). Industrial applications may include use as a solvent/carrier/encapsulating agent, moisture control agent, texturizer, and appearance control agent for substances other than colors and color modifiers, as well as a material handling aid. RPXG has a very stiff double helix structure in dispersion, which retains stability to heat, acid, and alkali. RPXG is more free flowing (*i.e.*, pourable) on application, but more viscous when settled; a highly desirable characteristic in liquid food systems. Typically, acetyl groups stabilize the ordered helix, while pyruvate groups destabilize the helix. The molecular weight is on the order of 2,000 kilodaltons. Xanthan gums that have the most pyruvic acid content tend to have higher viscosities and lower thermal stabilities, and reductions in pyruvic acid reduces the viscosity of the gum (Hassler and Doherty, 1990).

Table 4. Physical and chemical properties of reduced-pyruvate xanthan gum

Characteristic	Value	Reference
Appearance	Cream-colored	CP Kelco (2006a)
Arsenic	Not more than 3 mg/kg	Burdock (1997)
Assay	A sample yields, on the dried basis, not less than 4.2% and not more than 5.4% of carbon dioxide (CO_2), corresponding to between 91.0% and 117.0% of Xanthan Gum	FCC (2003)
Molecular weight	Approximately 2,000 Kilodaltons	Kelco Company (1975)
Isopropyl alcohol	Not more than 0.075%.	FCC (2003)
Lead	Not more than 2 mg/kg	FCC (2003)
Loss on drying	Not more than 15.0%	FCC (2003)
Physical form	Powder	FCC (2003)
Pyruvic acid	Less than 1.5%	FCC (2003)
Solubility	Readily dissolves in water with stirring to give highly viscous solutions at low concentrations	FCC (2003)
Viscosity	Passes test	FCC (2003)

FCC = Food Chemicals Codex

Pyruvate is a naturally occurring substance in the mammalian body as an end product in the metabolism of sugar or starch. Pyruvate is formed during glycolysis for the formation of adenosine triphosphate (ATP). From the glycolysis pathway, pyruvate may be processed through the oxidative Krebs cycle, producing energy in the form of guanosine triphosphate (GTP),

reduced nicotinamide adenine dinucleotide (NADH), and reduced flavin adenine dinucleotide ($FADH_2$), *via* oxidative reactions, ultimately released as carbon dioxide. Reduced FAD and reduced NAD donate energy from the Krebs cycle to cytochrome enzymes for the production of ATP. In non-oxidative reactions, pyruvic acid is processed through lactate dehydrogenase to form lactate and NAD^+. Pyruvate is not an essential nutrient; therefore, lack of pyruvate is not associated with a deficiency state (Graham Solomons, 1984; Brody, 1999a; Brody, 1999b).

3.2. Manufacturing Process and Specifications

Reduced-pyruvate xanthan gum production is comparable to that from the current xanthan gum production strain (*Xanthomonas campestris* XWCM-1) cultured under typical xanthan gum production conditions. A diagram of the basic manufacturing process is given in Figure 4. No variations in the fermentation conditions and the media constituents are necessary. The production of reduced-pyruvate xanthan gum is initiated by the fermentation of a nonpathogenic/nontoxigenic strain of *X. campestris* bacteria producing RPXG that conforms to product specifications. For example, *X. campestris* strain ATCC 31313 has been utilized to produce RPXG (Table 5). The nature and amounts of impurities in the finished xanthan gum preparation depend on the organism from which the product is produced, as well as the fermentation materials and methods used to grow the production organism. The impurities in the reduced-pyruvate xanthan gum preparation do not differ significantly from those in Keltrol®[7] xanthan gum, which conforms to FCC specifications (Table 5), as noted in the specifications for production of the two forms of xanthan gum (Table 5).

In a typical fermentation, *X. campestris* is initially cultured in a small flask containing a fermentable sugar, *e.g.*, glucose, sucrose or corn syrup, with organic and inorganic nitrogen sources, phosphate buffer and divalent cations in a batch fermentation process (Figure 4, Step 1). This allows for cell cultures to develop to a high enough concentration to inoculate a production-size seed tank fermentor. A seed tank is then filled with the similar nutrients for optimum gum production, and the flask culture is added. After expansion of the *X. campestris* cells (Figure 4, Step 2), the seed tank is emptied into a final fermentor tank, where the cells are fermented to maximize gum production (Figure 4, Step 3). The source sugars and proteins are exhausted and the gum yield is maximized by approximately 48 hours of fermentation. The final fermentation broth is pasteurized, usually through a heat exchanger that allows residence time to ensure microbial inactivation (Figure 4, Step 4). A high conversion of carbohydrate to xanthan gum results in gum concentrations well in excess of cellular dry weight. The resulting gum is mixed with two volumes of isopropanol to precipitate the polysaccharide from the broth (Figure 4, Step 5). The gum is mechanically compressed into a cake, removing the water, and the cake is then dried into a coarse fiber, sent into milling systems that grind the fibers into a particulate powder (Figure 4, Step 6). This powder is then screened for particle size, blended, and packed into final containers for shipment. The final product is tested for quality against purity specifications before shipment release (Figure 4, Step 7). If quality specifications are not reached, the product is destroyed or reformulated for industrial use.

Excluding pyruvate levels, reduced-pyruvate xanthan gum retains all of the chemical properties inherent in xanthan gum, including those set for xanthan gum identification purposes

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[7] The commercial xanthan gum produced by CP Kelco.

and in the specification limits for xanthan gum, as set forth in the *Food Chemicals Codex* monograph for xanthan gum and in federal regulations (FCC, 2003).[8]

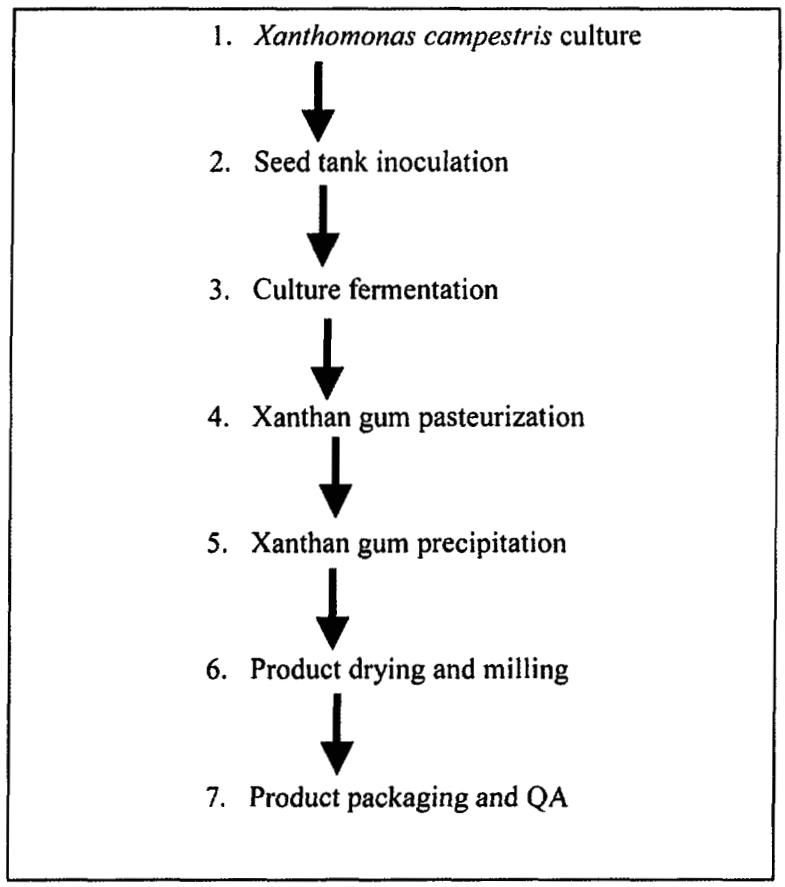

Figure 4. Manufacturing process of reduced pyruvate xanthan gum (Kelco Company, 1975)

3.3. Stability

Xanthan gum is stable for at least two years at 4°C (some minimal degradation occurs after two years of storage at room temperature). The decrease in pyruvate content in RPXG is not expected to alter this level of stability (CP Kelco, 2006e).

[8] Title 21 of the US Code of Federal Regulations (CFR), section 172.695, 2005

Table 5. Regulatory specifications for commercial xanthan gum (Keltrol®)[9] and test results of RPXG xanthan gum (CP Kelco, 2006c)

Identification Tests	FDA 21 CFR 172.695	FCC (2003)	USP 28/NF 23 (2005)	Reduced-Pyruvate Xanthan Gum (RPXG)	Keltrol®-05-412 XWCM-1	RPXG -05-407	RPXG-05-409	RPXG -05-410	RPXG -05-411	RPXG -05-697
Ash (%)			6.5 – 16.0	Not more than 16.0	10.1	5.0	4.4	4.2	6.7	6.6
Assay* (%)		91.0-117.0, dried basis	91-108, dried basis	91.0-117.0, dried basis						
Locust Bean Gum Gel Test	Positive	Pass	Pass	Positive/Pass	Pass	Pass	Pass	Pass	Pass	Pass
1% Potassium chloride Viscosity (cP)	Not less than 600	Not less than 600	Not less than 600	Not less than 600	1,670	1,500	1,750	1,570	1,600	1,790
Pyruvic Acid (%)	More than 1.5	Not less than 1.5	Not less than 1.5	<1.5	5.34	0.42	0.43	0.33	0.31	0.20
Viscosity Ratio	1.02 – 1.45	1.02 – 1.45	NR	1.02-1.45	1.23	1.42	1.20	1.09	1.16	1.09
Additional Limits										
Arsenic (ppm)	NA	Not more than 3		<3	<3.0	<3.0	<3.0	<3.0	<3.0	<3.0
Carbon Dioxide (%)	NA	4.2 to 5.4	4.2 – 5.0	4.2 to 5.4	4.9	4.8	4.9	4.9	4.9	4.9
Heavy Metals (ppm)	NA	Not more than 20	Not more than 30	Not more than 20	1.6	3.4	1.7	1.2	1.4	2.5
Isopropyl Alcohol (ppm)	Not to exceed 750	Not more than 750	Not more than 750	Not more than 750	510	320	560	430	670	360
Lead (ppm)	NA	Not more than 2		<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0
Loss on Drying (%)	NA	Not more than 15.0	Not more than 15.0	Not more than 15.0	7.1	7.2	7.7	7.2	6.9	5.6

cP = centipoise; FCC = Food Chemical Codex; NA = not applicable; NR = not reported; ppm = parts *per* million; RPXG = reduced pyruvate xanthan gum; USP = United States Pharmacopeia; *A sample yields, on the dried basis, not less than 4.2% and not more than 5.4% of carbon dioxide (CO_2), corresponding to between 91.0 and 117.0% of xanthan gum (FCC, 2003)

[9] Name of commercial gum produced by CP Kelco

4. PROPOSED AND CURRENT USES

Reduced-pyruvate xanthan gum, when produced from a nonpathogenic/nontoxigenic *Xanthomonas campestris* strain of bacteria under typical fermentation conditions, as exemplified by RPXG production by a variant strain of *X. campestris* (ATCC 31313), is proposed for food use(s) identical to that currently in place for xanthan gum in commerce (ATCC, 2005). Xanthan gum solutions are extremely pseudoplastic, possessing extremely high viscosities when not under the influence of shear forces. This contributes to the effective stabilizing abilities of xanthan gum (Clegg, 1996). Reductions in the pyruvic acid content of xanthan gum reduces the viscosity and slightly alters its rheological properties, increasing the suspending capacity of the reduced-pyruvate xanthan gum in solution. Current food uses include use as a stabilizer, emulsifier, thickener, suspending agent, bodying agent, or foam enhancer in foods for which standards of identity established under section 401 of the Act do not preclude such use.[10] Application levels of RPXG are, in general, the same as for XG.

The US Food and Drug Administration Center for Drug Evaluation and Research (CDER) maintains a list of inactive ingredients in drugs (CDER, 2004). In this list, xanthan gum is reportedly used in the following oral delivery forms: tablet; tablet, coated for sustained action; drops; granule; granule for suspension; powder; powder for reconstitution; powder for oral suspension; suspension; suspension for sustained action; and as an orally disintegrating tablet. Xanthan gum is also reportedly used in enema formulations and for topical administration in emulsions and lotions, and as an excipient in oral and topical pharmaceutical formulations and cosmetics. Although primarily used as a suspending agent, xanthan gum has been used to prepare sustained-release matrix tablets (Kibbe, 2000).

4.1. Consumption

As xanthan gum used in both foods and drugs must be of food grade (industrial applications are exempted from food grade status), the annual poundage (*i.e.*, volume) reported by manufacturers as that amount disappearing from the market (200,177,680 pounds) in 2001 likely includes all xanthan gum consumed by humans (PAFA, 2006). In the unlikely event that all xanthan gum is replaced by RPXG, the consumption of xanthan gum will be calculated for a safety determination of RPXG. To calculate the amount of xanthan gum consumed on a mg/kg body weight/day basis, the market disappearance of xanthan gum (200,177,680 pounds) in 2001 was divided by the 2000 US census population of 281,421,906[11] to determine the xanthan gum *per capita* intake of 0.7713 pounds *per* person *per* year. Dividing by 365 days *per* year and converting to milligrams results in a daily intake of 883.9560 mg/person/day xanthan gum. Based on an average weight of 60 kg, the average daily consumption of xanthan gum is stated at 14.7326 mg/kg body weight/day. It is likely that the actual value is less than this amount, as (1) the conservatism of the calculations, and (2) a substantial portion of xanthan gum is diverted to cosmetic and other dermal uses. Since xanthan gum is a large molecular weight polysaccharide, it is unlikely to be absorbed through the skin and therefore dermal application should not be considered a contributor to total body burden.

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[10] Title 21 of the US Code of Federal Regulations (CFR), section 172.695, 2005

[11] US Census Bureau, 2000 census. http://www.census.gov/main/www/cen2000.html; site visited on March 22, 2006.

Because RPXG produced by a variant strain of *X. campestris* results in a xanthan gum that is more stable in foods, the entry of reduced-pyruvate xanthan gum into the marketplace would not change consumption of xanthan gum. Therefore, the use of reduced-pyruvate xanthan gum would not increase the total amount of xanthan gum consumed, but would replace typical xanthan gum currently added to food products, and therefore the daily consumption of RPXG is stated at 14.7326 mg/kg body weight/day.

4.2. Methods for Detection in Food

The content of pyruvate in gums has previously been utilized as an identification of xanthan gum (FCC, 2003). Xanthan gum was found to be one of the few gums that, on average, contained substantial (*e.g.*, greater than 1.5%) levels of pyruvate. As RPXG produced under typical fermentation conditions conforms to all manufacturing specifications to commercial xanthan gum, except the regulatory standards for pyruvate content, other methods must be established to uniquely identify RPXG, when compared with other types of gums. Table 6 indicates that use of the brine solubility test, the pyruvic acid test, and the locust bean gum gel test, may be utilized to uniquely characterize reduced-pyruvate xanthan gum, when compared with other types of gum substances.

Table 6. Unique characteristics that identify reduced-pyruvate xanthan gum from other gums (CP Kelco, 2006d)

Food Additive (lot #)	Brine Test	Pyruvic Acid (%)	Locust Bean Gum Gel Test
Xanthan Gum (514287A)	Fail	4.55 %	Pass
Low Pyruvate Xanthan gum (05-686)	Pass	0.22 %	Pass
Gellan Gum (4K1465)	Fail	0.21 %	Fail
CMC (NSP42112)	Fail	0.16 %	Fail
Pectin (S53003)	Fail	1.07 %	Fail
Carrageenan (S52554)	Fail	1.83 %	Fail

4.2.1. Characterization methods.

4.2.1.1. Brine-Solubility test

Add 253 ml water to a stainless steel mixing cylinder. Begin stirring at approximately 11,500 revolutions *per* minute (rpm). Add 161 g anhydrous calcium chloride. Maintain caution, as the solution gets warm. Stop mixing after five minutes and remove the cylinder. Scrub down the container wall to dislodge any adhering salt into the brine, using a plastic spatula. Insure that all the material is returned to the fluid portion. Allow the solution to cool to between 42°C and 44°C. Return the cylinder to the mixer, and continue mixing the solution at approximately 11,500 rpm. Slowly add 0.86 g sample of gum to the brine over a 15-30 second period and stir the solution for five minutes. Using a plastic spatula, scrub down the container wall to dislodge any adhering polymer. Insure that all this material is returned in the fluid. Place the cylinder back on the mixer and continue mixing for 40 minutes. At the end of the 40-minute period, add two drops of 1-octanol and allow the mixing to continue for 30 seconds. Cool the sample and

cylinder to lower the fluid temperature to 25 ± 1°C. The reduced-pyruvate xanthan gum will form a homogeneously thickened fluid. Record the sample as "pass" for reduced-pyruvate xanthan gum if a homogeneously thickened fluid forms. Record the sample as "fail" for reduced-pyruvate xanthan gum if a thickened fluid does not form (CP Kelco, 2006b).

4.2.1.2. Pyruvic acid test

Pipet 10 ml of an 0.6% solution of the polysaccharide in distilled water (60 mg of a water-soluble gum) into a 50 ml flask equipped with a standard taper glass joint. Pipet in 20 ml of 1N hydrochloric acid. Weigh the flask. Reflux the mixture for three hours. Take precautions to avoid loss of vapor during the refluxing. Cool the solution to room temperature. Add distilled water to make up any weight loss from the flask contents. Pipet 1 ml of a 2,4-dinitro phenyl hydrazine reagent (0.5% in 2N hydrochloric acid) into a 30 ml separatory funnel followed by a 2 ml aliquot (4 mg of water-soluble gum) of the polysaccharide hydrolyzate. Mix and allow the reaction mixture to stand at room temperature for five minutes.[12] Extract the mixture with 5 ml of ethyl acetate. Discard the aqueous layer. Extract the hydrazone from the ethyl acetate with three 5 ml portions of 10% sodium carbonate solution. Dilute the combined sodium carbonate extracts to 100 ml with additional 10% sodium carbonate in the 100 ml volumetric flask. Measure the optical density of the sodium carbonate solution at 375 millimicrons. Compare the results with a curve of the optical density versus concentration of an authentic sample of pyruvic acid that has been run through the procedure starting with the preparation of the hydrazone. Record the *percent* by weight of pyruvic acid in the test polysaccharide. Note "positive" for xanthan gum if the sample contains more than 1.5% of pyruvic acid and "negative" for xanthan gum if the sample contains less than 1.5% of pyruvic acid by weight (FDA, 2005).

4.2.1.3. Locust Bean Gum Gel Test

Blend on a weighing paper or in a weighing pan 1.0 g of powdered locust bean gum with 1.0 g of the powdered polysaccharide to be tested. Add the blend slowly (approximately ½ minute) at the point of maximum agitation to a stirred solution of 200 ml of distilled water previously heated to 80°C in a 400 ml beaker. Continue mechanical stirring until the mixture is in solution, but stir for a minimum time of 30 minutes. Do not allow the water temperature to drop below 60°C. Set the beaker and its contents aside to cool in the absence of agitation. Allow a minimum time of two hours for cooling. Examine the cooled beaker contents for a firm rubbery gel formation after the temperature drops below 40°C. In the event that a gel is obtained, make up a 1% solution of the polysaccharide to be tested in 200 ml of distilled water previously heated to 80°C (omit the locust bean gum). Allow the solution to cool without agitation as before. Formation of a gel on cooling indicates that the sample is a gelling polysaccharide and not xanthan gum. Record the sample as "positive" for xanthan gum if a firm, rubbery gel forms in the presence of locust bean gum but not in its absence. Record the sample as "negative" for xanthan gum if no gel forms or if a soft or brittle gel forms both with locust bean gum and in a 1% solution of the sample (containing no locust bean gum) (FDA, 2005).

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[12] A hydrozone is formed with the pyruvate and the hydrazine.

5. ABSORPTION, DISTRIBUTION, METABOLISM AND ELIMINATION (ADME)

Typical fermentation procedures normally result in the production of xanthan gum that has been known to contain variations in the percentage of pyruvate contained within the gum (Cadmus *et al.*, 1976; Sandford *et al.*, 1977; Slodki and Cadmus, 1978). The percentage of pyruvate typically found in currently manufactured xanthan gum products ranges between 4-6% of the total gum (Cadmus *et al.*, 1976), making pyruvate a minor constituent in xanthan gum. Variations in the fermentation characteristics, such as reduced oxygen supply, magnesium concentrations and potassium concentrations also results in xanthan gum with reduced pyruvic acid levels (Jarman and Pace, 1985; Peters *et al.*, 1993). The removal of pyruvate does not change the structure or functional properties of the gum (Tait *et al.*, 1986; Hassler and Doherty, 1990; Becker *et al.*, 1998). The significant alteration to the xanthan gum is the reduction in the *percentage* of the pyruvate molecule attached to the carbohydrate structure. No other addition or modification of the structural components of xanthan gum has been altered. Considering that the reduced-pyruvate xanthan gum is produced by the same manufacturing processes as currently marketed xanthan gum, and a reduction in the pyruvate content of xanthan gum does not change the overall structure of the gum, xanthan gum lacking substantial amounts of pyruvate is substantially equivalent to that currently found in commerce. Therefore, safety studies of xanthan gum containing pyruvate may be reviewed for a safety determination of reduced-pyruvate xanthan gum.

Xanthan gum is a food ingredient composed of β-D-glucosyl, α-D-mannosyl, β-D-mannosyl, and β-D-glucosyluronic acid residues and differing proportions of acetyl and pyruvate groups. The primary structure consists of a cellulose backbone with trisaccharide side chains, with a pentasaccharide repeating unit. Digestibility and caloric availability studies indicate the body does not utilize that xanthan gum, as Booth *et al.* (1963) found that practically all of the gum fed during a seven-day period could be accounted for in the feces. A summary by JECFA of an unpublished study described *in vitro* tests that indicated nonenzymatic hydrolysis and colonic microorganism fermentation as responsible for the initial breakdown of xanthan polysaccharide *in vivo*. ^{14}C-Labeled xanthan gum, prepared by fermentation of uniformly labeled glucose with *Xanthomonas campestris*, was also fed to rats at a level of 2% (50 mg total) in the diet (JECFA, 1987). Results indicated that a maximum of 15% of the label was metabolized to carbon dioxide in 100 hours. Acetate and pyruvate accounted for only 9.8% of the label in the gum used. No accumulation in the tissues was found, and the metabolism of the labeled material and the distribution of ^{14}C in tissues were to be expected from feeding a simple ^{14}C-labeled molecule, such as a hexose or acetate. Approximately 98% of the fecal radioactivity was attributed to unchanged or only slightly modified polysaccharide. No other data was provided in the summary.

Sunvold *et al.* (1995) determined the fermentability of selected fibrous substrates by dog fecal microflora. Freshly voided dog fecal samples were processed and inoculated with 310 mg of polysaccharide or blank, resulting in a substrate concentration of 10 mg/ml. The tubes were incubated at 39°C for 6, 12, or 24 hours, then analyzed for short chain fatty acid production and organic matter disappearance. Xanthan gum, oat fiber, gum karaya and Solka floc®[13] produced

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[13] Solka floc® is a highly purified functional cellulose, composed of *beta*-1,4-glucan units. http://www.ifcfiber.com/products/solkafloc.php; site visited December 14, 2005.

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the least amount of total short-chain fatty acids ($P<0.05$). The organic matter disappearance was lowest for xanthan gum, Solka Floc®, gum karaya, and gum Arabic ($P<0.05$). In addition, xanthan gum produced significantly less butyrate, propionate and acetate concentrations ($P<0.05$). This study indicates that xanthan gum is not readily metabolized by dog fecal microflora.

6. SAFETY EVALUATION

Xanthomonas campestris is a ubiquitous soil microorganism not known to be toxigenic or pathogenic for animals or humans. A search of the scientific literature to December, 2005 did not reveal any reports of any animal or human toxigenicity or pathogenicity attributed to any strain of *Xanthomonas campestris*. Further evidence of the non-pathogenic nature of *Xanthomonas campestris* is the fact that the optimum growth temperature is 27-31°C, with no growth at 37°C (human body temperature) (ATCC, 2005). Booth *et al.* (1963) examined the effects of xanthan gum in rats. Xanthan gum fed at a dietary level of 15% produced abnormally large fecal pellets and reduced the food intakes and growth rates, which may be due to decreased nutrient intake from an abnormally large intake of a macronutrient (Borzelleca, 1992). In addition, a paired-feeding study compared the growth of rats ingesting *ad libitum*, a diet containing 7.5% xanthan gum (approximately 3750 mg/kg/day), with a basal diet reduced in caloric intake by 7.5% (Booth *et al.*, 1963). After 18 days on the diets, the rat weight gains were identical for the restricted and *ad libitum* xanthan gum groups, indicating both the absence of any growth-inhibiting factor in xanthan gum and that xanthan gum is not utilized as a source of calories. In summary, this information indicates that the main constituent is the carbohydrate fraction of the xanthan gum molecule, which is not utilized by the body (Booth *et al.*, 1963). The reduced-pyruvate xanthan gum is essentially equivalent to commercially produced xanthan gum. As such, relevant studies discussing the safety and possible adverse effects of xanthan gum will be discussed.

6.1. Acute and Short-Term Toxicity Studies

Booth *et al.* (1963) evaluated the acute toxicity of xanthan gum administered *via* intraperitoneal or gavage dosage regimens in mice. Adult mice were administered (*via* gavage) 1 g xanthan gum/ kg bw[14] (higher doses could not be given due to increased viscosity). No effect of the treatment was observed. Xanthan gum was injected intraperitoneally into mice daily for two 5-day periods (separated by two days). Each injection consisted of 10 mg/ml xanthan gum in water. Abdominal swelling was noted during the injection period, but no deaths occurred during the treatment period, and up to two weeks after the final injection. Xanthan gum was not observed in the abdominal cavity at autopsy, which suggested that the injected xanthan gum was absorbed (Booth *et al.*, 1963).

A 5% aqueous solution of xanthan gum applied daily as a topical to shaved areas of rabbit skin caused localized irritation, followed by cracking of the skin and bleeding (Booth *et al.*, 1963). The control (water-soluble cornstarch) solution produced similar effects. Rapid healing occurred when the applications were discontinued.

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[14] bw = bodyweight.

Trout *et al.* (1983) evaluated the effects of *ad libitum* feeding of xanthan gum on potential distention of the gastrointestinal tract, and to determine if the distention of the gastrointestinal tract was due to the extra amount of material consumed, or an increased intestinal cell mass. The research team also determined if the additional digestive tract contents would be due to extra water or extra dry matter, and if retarded absorption of simple sugars accounted for the extra water in the small intestine. Male Wistar rats (six or eight rats *per* group) were fed nutritionally adequate diets containing either 4% xanthan gum or cellulose (approximately equivalent to 2000 mg/kg/day). Maltose was provided in place of glucose in order to reduce hypertonicity of the ingestate and the possibility of osmotic insult in the proximal digestive tract. The diets were administered for seven days, with the rats sacrificed eight to eleven hours into the dark period of Day 8, to evaluate the fullness of the digestive tract during a typical nocturnal feeding pattern.

The wet and dry weights of contents from the stomach, large intestine cecum, and segments of the small intestine were analyzed, as well as the wet and dry weight of tissue in these portions of the digestive tract. The quantities of simple sugars in successive portions of the small intestine were also analyzed enzymatically for total glucose.

Xanthan gum feeding increased the dry weight of tissue mass of the small intestine, but not of the stomach, cecum, or large intestine, while the food intake remained similar to control values. Xanthan feeding increased the combined weight of the small intestine and its contents by 110%, which contained 36% more wet cell mass than did controls, accounting for 23% of the total difference in intestinal weights. There was a 400% increase in the water in the intestinal contents, relative to controls, which accounts for more than 70% of the gross difference. In addition, xanthan feeding did not significantly increase the sugar content found in the proximal quarter of the small intestine, but actually prevented the sharp decline in sugar contents that was observed in the cellulose-fed controls. Xanthan gum increased the sugar recovered in the segments of small intestine by 150%, compared with cellulose-fed rats. Xanthan feeding did not affect the amount of diet eaten during the eight to eleven hours of the final dark period or the amount of dry matter recovered in the stomach. This and other studies indicate that xanthan gum does not increase the amount of calories absorbed into the system, and actually retains sugars and water in the intestinal system (Trout *et al.*, 1983; Ou *et al.*, 2001).

A two-week oral feeding study was conducted to determine the relationship between the viscosity of indigestible polysaccharides and the function of digestive organs (Ikegami *et al.*, 1990). Male Sprague-Dawley rats (five weeks of age) were given *ad libitum* access to water and food. The diet consisted of the addition of 5% sucrose (control diet) or 4.3% to 4.9% of dietary fiber, in the form of xanthan gum (approximately 4300-4900 mg/kg bw/day), apple pectin, *alpha*-carrageenan, locust bean gum, guar gum, sodium alginate, alginic acid, or calcium alginate (experimental diet). The diet was administered for two weeks, after which the animals were fasted overnight, given approximately 4 g of their respective diets and sacrificed six hours later. The digestive tract and pancreas were removed and examined for adverse effects.

After two weeks of consumption, the final body weights of the xanthan gum-fed rats were not altered from controls. The administration of any of the indigestible viscous polysaccharides increased the weight and length of all digestive organs ($P<0.05$). The enlargement of the digestive organs and pancreas was caused by xanthan gum, sodium alginate,

locust bean gum, and guar gum. These indigestible gums also increased the volume of secreted pancreatic juice and the output of amylase, protease and total bile acids ($P<0.05$). However, the final body weight after a two-week consumption of the indigestible polysaccharide xanthan gum was not altered from control values (Ikegami *et al.*, 1990), indicating a lack of adverse effects of consumption of high levels of xanthan gum.

Cameron-Smith *et al.* (1994) investigated the action of the gastrointestinal tract on the viscosity of several viscous fiber types, including xanthan gum. The viscous fibers (*i.e.*, xanthan gum, guar gum and methylcellulose) were fed to male Sprague-Dawley rats (five *per* group) at a dose of 70 g xanthan gum *per* kg of food (approximately equal to 3500 mg/kg bw/day). A non-viscous wheat-bran was utilized as a control. The diet was fed *ad libitum* for two weeks, after which the rats were sacrificed and the stomach and small intestinal digesta were collected for viscosity measurement. No adverse effects were noted in any of the rats administered the test diets. Supplementation of the diet with xanthan gum, guar gum, or methylcellulose resulted in thick, paste-like digesta. Viscosity measurements of the digesta of the various diets indicated that the viscosity of the xanthan diet was approximately 500-fold greater than that measured for the methylcellulose and guar diets.

In summary, xanthan gum has been found to lack acute oral toxicity in mice at 1000 mg/kg bw/day, the highest concentration dosed, due to viscosity issues, and intraperitoneal administration of 10 mg/ml xanthan gum for ten days did not result in adverse effects. Xanthan gum has been fed to rats at concentrations up to 3500 mg/kg bw/day, with a lack of adverse effects.

6.2. Irritation and Sensitization Studies

In an irritation study, a 1% aqueous solution of crude xanthan gum was applied to a shaved area of the backs of five adult male rats every third day for a total of 15 applications, and compared with a similar application of 2% gum guar (Hendrickson and Booth, 1965). There was no evidence of irritation or tissue damage during the application period, and up to two weeks after termination of the treatments. In a study evaluating the irritative effects of xanthan gum in the eye, 0.2 ml of a 1% xanthan gum solution was placed in the conjunctival sac of the left eye of two adult albino rabbits daily for five days (Hendrickson and Booth, 1965). The right eye served as a control, and was instilled with water. No evidence of irritation or damage was noted during the treatment or seven days following the fifth application.

In a guinea pig sensitization study, eight white male guinea pigs each received a series of ten intracutaneous injections at the rate of three *per* week on the shaved back or flank (Hendrickson and Booth, 1965). The injection consisted of 0.1 ml of 0.2% crude xanthan gum dissolved in 0.9% saline (a volume of 0.05 ml was administered for the first injection only). Two weeks after the tenth injection, a challenge injection was made using 0.05 ml of the xanthan gum solution. Twenty-four hours after the challenge injection, a comparison of the diameter, height, and color of the reaction was made with the average reaction measurements made after each of the original ten injections. The challenge injection produced erythema at the site, and a wheal that averaged 10 x 10 mm in diameter, and a height of 3 mm. In the initial injection series, there was an increasing local reaction after the second injection, which occurred 24 hours after each succeeding injection. By the tenth injection, the erythema and swelling was comparable to that produced that the subsequent challenging dose. Compared with a similar experiment evaluating

guar gum as a sensitizing agent, the results for both xanthan gum and guar gum were very similar. Therefore, the authors analyzed the gum preparations for nitrogen content, which was found to be 0.65 and 0.71% for xanthan and guar gum, respectively. The authors calculated that both of the gums contained in excess of four percent protein. Based on the high protein content, the authors concluded that the protein impurities in the crude gum preparations were responsible for any potential positive sensitization reactions (Hendrickson and Booth, 1965).

In a subsequent sensitization study, a weanling albino rabbit was administered a primary intramuscular injection of 50 mg crude xanthan gum containing adjuvant (*i.e.*, calcium alginate), followed with 25 mg crude xanthan gum injected at two-day intervals for three weeks (Hendrickson and Booth, 1965). The rabbit did not exhibit sensitivity to the xanthan gum injections. Serum was drawn and passive sensitization was attempted upon four guinea pigs by the passive cutaneous anaphylaxis (PCA) method. Each guinea pig was injected intradermally with 0.05 ml of a diluted serum solution (serum diluted 1:4 with isotonic saline). The guinea pigs were challenged with 10 mg crude xanthan gum in saline injected by the intravenous route. No passive reactions occurred in any of the guinea pigs. The author therefore concluded that xanthan gum was not highly antigenic in rabbits.

A study determining the relationship between the handling of xanthan gum powder (inhalation exposure) and reports of flu-like symptoms and/or hypersensitivity pneumonitis found that no acute or chronic effects from xanthan gum exposure on pulmonary function could be measured in a group of plant workers exposed to xanthan gum (0.1% to 1.9 mg/m^3) 60% to 100% of the time spent in the plant (Sargent *et al.*, 1990).

6.3. Subchronic Studies

A subchronic study evaluated the digestibility of xanthan gum in rats, as part of a study that also evaluated dextran B-512F, pectin, gum Arabic, agar, guar gum, and phosphomannan Y-2448 (Booth *et al.*, 1963). Albino rats (sex or age not stated) ingested diets containing 15% xanthan gum for 91 days. In addition, a seven-day study was conducted to evaluate the polysaccharides (7.5% xanthan gum *via* the diet) for their caloric availability and digestibility.

Xanthan gum consumption at a dietary level of 15% (approximately equivalent to 7500 mg/kg/day) produced abnormally large fecal pellets, which were well formed. Reduced food intakes and decreased growth rates were observed when 7.5-15% dietary levels of xanthan gum were consumed. At diets containing 3% or 6% xanthan gum (approximately equivalent to 1500 or 3000 mg/kg/day, respectively), weight gains were not significantly different from control levels. No significant alterations in hemoglobin or red and white cell counts, or organ weights, were observed in rats consuming 3% or 6% xanthan gum. No evidence of pathology was noted from histologic examination of the tissues of rats that had ingested 15% xanthan gum for 91 days. Evaluation of weight gains and fecal amounts indicated that rats consuming xanthan gum lacked a significant net change in weight, comparable to basal controls. The authors concluded that xanthan gum is not utilized, which was substantiated by the fact that practically all the xanthan gum fed during a seven-day period could be accounted for in the feces (Booth *et al.*, 1963).

In a similar study, xanthan gum was fed to young adult beagle dogs (n = 2 dogs *per* sex *per* group) at 1000 and 2000 mg/kg/day for twelve weeks (Robbins *et al.*, 1964). Another group

of dogs were fed 2000 mg/kg/day cellulose as a control group, with a fourth group that did not receive supplementation (negative control). Control and treated groups lost weight during the treatment period, with weight loss being greater for the high-dose xanthan gum group (statistical significance was not determined). The authors stated that the only adverse effect noted in the general condition of the animals was an immediate and persistent diarrhea in the high dose xanthan gum group. The dogs fed 2000 mg/kg/day xanthan gum had decreased red blood cell counts and hemoglobin levels, which the authors concluded was due to the constant diarrhea of this dose group. All liver and kidney function values were within normal limits. Serum cholesterol was reduced in both the cellulose and xanthan gum-treated animals, with the greatest decrease in the high dose xanthan gum group. At autopsy no gross defects were noted, other than three small cysts and/or inflammation in the reproductive system of three control dogs. No inflammation of the intestinal mucosa was observed (Robbins *et al.*, 1964). A NOAEL of 2000 mg/kg/day may be stated for xanthan gum in dogs.

In summary, xanthan gum consumption by rats for 91 days at a dose of approximately 7500 mg/kg bw/day resulted in nonsignificant decreases in food intake and growth rates, when compared to controls. Xanthan gum ingestion by dogs for 84 days at a dose of 2000 mg/kg/day resulted in diarrhea and nonsignificant decreases in body weight, red blood cell counts and hemoglobin levels, when compared to controls. Xanthan gum was determined to be indigestible in both the rat and dog. From this data, a NOAEL of 7500 mg/kg/day is posited.

6.4. Chronic Studies

Xanthan gum has been tested in two-year feeding studies in rats and dogs (Woodard *et al.*, 1973). In the two-year rat study, four groups of 30 male and 30 female weanling Charles River CD strain rats were administered xanthan gum at levels in the basal diet to provide daily dosages of 0, 250, 500, and 1000 mg/kg/day. Hematological analysis was conducted on five male and five female rats from the control and 1000 mg/kg/day dose groups throughout the study, and five male and five female rats from each level at the study termination. Gross necropsies were performed for all rats that survived, as well as for any found dead or sacrificed in moribund condition. Tissues examined microscopically from approximately 50 rats from each dose group included adrenal glands, bone marrow, brain, colon, eye, gonads, heart, kidney, liver, lung, lymph node, mammary glands, pancreas, pituitary, skeletal muscle, small intestine, spleen, stomach, thyroid, urinary bladder, and uterus or prostate.

Treated and control rats showed comparable survival, with no significant differences in body weight gain. Hematologic and biochemical values in xanthan gum-fed rats were not significantly different from control values throughout the study, except at termination when hemoglobin and hematocrit values were low (nonsignificant) for two of five males in the 1000 mg/kg/day dose group. The incidence of spontaneous diseases that are commonly found in aging rats did not vary between xanthan gum and control dose groups. Soft stools were noted more frequently for the high and middle level xanthan gum males than for control males, although this effect was not statistically significant. No significant differences were noted between xanthan gum-treated rats and controls. Therefore, within the conditions of study described above, NOAEL of 1000 mg/kg bw/day is established (Woodard *et al.*, 1973).

A two-year dog feeding study administered xanthan gum to groups of four male and four female Beagle dogs, at daily basal levels calculated to yield 0, 250, 370, and 1000 mg/kg/day,

seven days *per* week, for 107 weeks (Woodard *et al.*, 1973). All dogs were sacrificed after 107 weeks and subjected to gross necropsy.

Blood pressure, electrocardiograms, behavior, body weights, gross and microscopic examination of tissues, and absolute and relative organ weights were similar for control and xanthan-treated dogs. The weight of feces excreted increased dose-dependently in xanthan gum-treated dogs. The average weight of feces excreted each day was 138, 200, 273, and 289 g from control, 250, 370, and 1000 mg/kg/day xanthan gum-treated dogs, respectively. The authors indicated that this increased weight was most likely due to the known ability of xanthan gum to retain water (Woodard *et al.*, 1973). This study indicates that xanthan gum fed to dogs at doses up to 1000 mg/kg/day does not result in adverse events.

In summary, chronic (two year) administration of xanthan gum at doses up to 1000 mg/kg bw/day to rats and dogs did not result in any toxicologically significant findings for the parameters tested, albeit the studies were not conducted according to currently accepted (*i.e.*, FDA Redbook) standards. Based on these data, a NOAEL of 1000 mg/kg/day is established in rats and dogs.

6.5. Reproduction Studies

For evaluation of reproductive effects in the rat, xanthan gum was administered *via* the diet to groups of 10 male and 20 female albino rats of the first generation (F_0) and to groups of 20 male and 20 female albino rats of the two successive generations (F_1 and F_2), at levels providing daily doses of 0, 250, and 500 mg/kg body weight/day xanthan gum (Woodard *et al.*, 1973). Pairs of rats from the F_0, F_1 and F_2 generations were mated to produce two litters *per* generation (a and b) with the next generation selected from weanlings of the previous litters, resulting in F_{1a} and F_{1b}, F_{2a} and F_{2b}, and F_{3a} and F_{3b} litters, respectively (Table 7).[15]

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[15] In a typical generational reproductive study, agent-exposed female animals (F_0) are bred to untreated, control males with the production of F_{1a} litter that are euthanized at birth for morphological examination for abnormalities. The F_0 females are rested for two weeks while continuing the exposure, then rebred with untreated males to produce F_{1b} litters which the treated females rear. Females selected from the F_{1b} litters are bred with untreated control male animals to produce the next (F_2) generation. The females from the F_2 generation are bred with untreated control males, for the subsequent F_2 litters (F_{2a} and F_{2b}, respectively). This process is continued for the F_3 generation (Derelanko and Hollinger, 2006).

Table 7. Three generation reproduction study on rats fed xanthan gum in the diet (Woodard *et al.*, 1973)

Diet level (mg/kg)	Females mated	Litters	Live births	Stillbirths	Litter size	Birth Weight (g)	% Alive weaning	Weanling weight (g)[a]
				F_1a Generation				
0	20	16	134	3	8	5.7	91.8	29.0
250	20	14	132	9	9	6.0	60.6	29.2
500	20	17	157	0	9	5.9	82.2	32.1
				F_1b Generation				
0	19	16	132	1	8	6.9	81.8	33.7
250	18	15	133	0	9	7.1	90.2	34.7
500	19	18	172	2	9	6.6	88.4	31.3
				F_2a Generation				
0	20	17	165	13	10	6.1	69.7	31.7
250	21	17	181	5	11	6.1	62.4	29.9
500	22	21	210	4	10	6.1	86.7	33.0
				F_2b Generation				
0	19	13	140	4	11	6.1	71.4	34.0
250	20	19	214	7	11	6.3	78.0	31.4
500	22	21	237	7	11	2.9	74.7	34.2
				F_3a Generation				
0	22	16	129	2	8	6.1	78.0	36.7
250	22	18	158	2	9	6.2	66.0	30.7
500	22	16	139	14	9	5.9	70.0	33.1
				F_3b Generation				
0	22	18	157	12	9	6.6	85.0	41.2
250	22	16	156	1	10	6.7	67.9	36.3
500	22	18	159	6	9	6.4	76.1	41.5

[a]Mean values

The litters were evaluated for the number of litters *per* group, numbers of live births and stillbirths, physical condition, mean weights at birth and weaning, and *percent* young alive at weaning. Also evaluated were gross autopsy observations on litters of the second and third generations, malformations of offspring, body, kidney, heart, and liver weights on weanlings of the second (F_3b) litters of the third generation, and histopathological observations on tissues of weanlings in each control and high level (500 mg/kg/day) F_3b litter.

No differences were noted between xanthan gum and control rats in survival, general appearance, and reproductive performance. When compared with controls, xanthan gum-treatment lacked an effect on number of litters *per* group, numbers of live births, physical condition, mean weights at birth and weaning, *percent* young alive at weaning, and gross autopsy observations. Females of the F_0 and F_1b generations that had fewer than two litters were examined for uterine implantation sites, which revealed no effect by xanthan gum administration. No malformations were observed in any of the offspring. In summary, dietary feeding of xanthan gum at 250, or 500 mg/kg/day to groups of albino rats of both sexes during a three-generation reproduction study exhibited no differences from controls of the same generation (Woodard *et al.*, 1973).

6.6. Observations in Humans

Five male volunteers, following a seven-day control period, consumed a dose of xanthan gum equal to 15 times the acceptable daily intake (ADI) in place at study initiation (the ADI was stated at 10 mg/kg body weight, although this has since been changed to "Not Specified"),[16] on each of 23 consecutive days (Eastwood *et al.*, 1987). The volunteers consumed between 10.4 g - 12.9 g xanthan gum daily (based on their individual weight), in three measured portions at intervals each day. Parameters monitored included fecal wet and dry weight, intestinal transit time, plasma biochemistry, hematology, urinalysis, glucose tolerance and insulin tests, serum immunoglobulins, triglycerides, phospholipids, HDL cholesterol, as well as breath hydrogen and breath methane levels.

Ingestion of xanthan gum acted as a bulking agent in terms of its effects on fecal wet and dry weight and intestinal transit time. The only effect on the measured endpoints was a moderate (10%) reduction in serum cholesterol and a significant increase in fecal bile acid concentrations. The pattern of blood glucose tests and plasma insulin remained normal for all subjects. Throughout the glucose tolerance tests, breath hydrogen excretion remained below 16 ppm and there was no change in breath methane production. As colonic fermentation is the only source of breath hydrogen, hydrogen measured in the breath can be related to undigested carbohydrates (Behall *et al.*, 1998; Hallfrisch and Behall, 1999). This study indicates that ingestion of xanthan caused neither adverse dietary nor physiological effects in any of the subjects, and is not fermented by the colonic bacteria, decreasing the likelihood of any caloric benefit from the pyruvate incorporated into xanthan gum, or reduced-pyruvate xanthan gum *per se* (Eastwood *et al.*, 1987).

Xanthan gum (baked into muffins) was examined for its acceptability at a 12 g/day level in diets of diabetic and non-diabetic subjects (Osilesi *et al.*, 1985). Recently diagnosed borderline, type II diabetic (n=9) or non-diabetic (n=4) subjects in a free-living situation consumed either xanthan-containing (XCM) or xanthan-free (XFM) muffins in a placebo-controlled, six-week crossover study. The daily dosage of the gum, when administered, was 12 grams *per* day, in a total of six muffins (2 g/muffin), to be eaten throughout the day. This is equivalent to approximately 200 mg/kg bw/day (based on a 60 kg person).

The subjects reported an occasional feeling of fullness after consuming the xanthan gum-containing muffins, but no diarrhea or severe gastrointestinal distress was noted. Consumption of xanthan gum for three or six weeks significantly (P<0.05 and P<0.01, respectively) decreased fasting serum glucose levels in diabetic and non-diabetic subjects, when analyzed as one population. When analyzed as two subpopulations, xanthan gum significantly decreased fasting and post-load serum glucose levels in diabetic subjects (P<0.05), while contributing to non-statistically significant decreases in control subjects. This study indicates that xanthan gum has been consumed at 200 mg/kg/day for up to six weeks, with no adverse effects noted.

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[16] An ADI without an explicit indication of the upper limit of intake ("ADI not specified") may be assigned to substances of very low toxicity, especially those that are food constituents or that may be considered as foods or normal metabolites in man. The expression "ADI not specified" has been adopted by the Joint FAO/WHO Expert Committee on Food Additives at its Eighteenth session. An additive having an "ADI not specified" must meet the criteria of good manufacturing practice; for example it should have proven technological efficacy and be used at the minimum level of efficacy, it should not conceal inferior food quality or adulteration, and it should not create a nutritional imbalance. http://www.inchem.org/documents/jecfa/jecmono/v08je01.htm; site visited on December 15, 2005.

7. EVALUATION

Reduced-pyruvate xanthan gum produced by *Xanthomonas campestris* is considered to be safe for food use and is substantially equivalent to the currently commercialized xanthan gum product, based on the following criteria:

- Reduced-pyruvate xanthan gum is made under the same manufacturing conditions as commercially produced xanthan gum, only utilizing a variant of the commercial *X. campestris* strain, and meets every limitation (*i.e.*, specifications for the locust bean gum gel test, 1% potassium chloride viscosity, the viscosity ratio, ash, *percent* carbon dioxide, loss on drying, arsenic levels, and isopropyl alcohol levels) set forth under 21 CFR 172.695 and in the Food Chemicals Codex monograph describing xanthan gum, except for a reduction in the amount of pyruvate.

- Reduced-pyruvate xanthan gum uses the same manufacturing process that renders it free of viable *Xanthomonas campestris* cells.

- Reduced-pyruvate xanthan gum has the same composition of sugar residues as the current commercial xanthan gum, having essentially the same stoichiometry with respect to the hexose units and molecular weight distribution, but with a reduced level of pyruvate.

Xanthan gum is approved for both direct and indirect food use, as set forth under 21 CFR 172.695. As a food ingredient, reduced-pyruvate xanthan gum will be used in accordance with current good manufacturing practice as a stabilizer, emulsifier, thickener, suspending agent, bodying agent, or foam enhancer in foods for which standards of identity established under section 401 of the Act do not preclude such use. To assure safe use of the ingredient, the label of its container shall bear, in addition to other information required by the Act, the name of the additive, *i.e.*, xanthan gum, and the designation of "food grade."

Digestibility and caloric availability studies indicate that the body does not utilize the cellulose backbone of xanthan gum, with the majority of administered xanthan gum accounted for in the fecal content. A small fraction of the gum (approximately 5%) may be digested by colonic bacteria. The pyruvate and acetyl constituents of xanthan gum are removed *via* hydrolysis and metabolized to carbon dioxide.

Xanthan gum has been found to lack toxicological effects when evaluated in acute and short-term studies in rats at levels up to 3500 mg/kg/day for fourteen days. Subchronic studies in rats fed up to 7500 mg/kg/day resulted in reduced food intake and decreased growth rates at this high dose level, although no effects were noted in a histological examination of the rat tissues that would indicate a concern for safety. No adverse effects were noted at 3000 mg/kg/day xanthan gum, when ingested for 91 days. Chronic studies in rats and dogs at doses up to 1000 mg/kg/day resulted in a lack of toxicological effects, although a dose-dependent increase in fecal weight was noted. A three-generation reproduction study in albino rats found that xanthan gum had no adverse effect on either the dams or the offspring at a dietary administration of up to 500 mg/kg/day. Human clinical studies indicate that consumption of xanthan gum is well tolerated, at

a dose of 200 mg/kg/day for up to six weeks, and caused neither adverse dietary nor physiological effects in human subjects.

In the unlikely event that RPXG replaces all currently used commercial xanthan gum for various food applications, RPXG would be consumed at a level of 14.73 mg/kg/day, less than a tenth of the level of xanthan gum that is well tolerated in human clinical studies.

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8. CERTIFICATION

The undersigned authors of this document—a dossier in support of GRAS status determination for food ingredient use of reduced-pyruvate xanthan gum—hereby certify that, to the best of their knowledge and belief, this document is a complete and balanced representation of available information, favorable as well as unfavorable, known by the authors to be relevant to evaluation of the substance described herein.

Ray A. Matulka, Ph.D. Toxicologist Burdock Group	14 JUN 06 Date
James C. Griffiths, Ph.D., D.A.B.T. Director of Toxicology Burdock Group	20 JUN 2006 Date

9. CONCLUSION

After critically evaluating the information available, the Expert Panel has determined that, based on common knowledge throughout the scientific community knowledgeable about the safety of substances directly or indirectly added to food, there is reasonable certainty that reduced-pyruvate xanthan gum (RPXG), produced in accordance with current Good Manufacturing Practice (cGMP), is substantially equivalent to xanthan gum currently deemed GRAS, and will not be harmful under the intended conditions of use, and is; therefore, Generally Recognized As Safe (GRAS), by scientific procedures, when used as an ingredient to provide effects as a stabilizer, emulsifier, thickener, suspending agent, bodying agent, or foam enhancer in foods for which standards of identity established under section 401 of the Code of Federal Regulations do not preclude such use. In particular, the Expert Panel has evaluated the proposed use of reduced-pyruvate xanthan gum at specified levels noted in this document and has concluded that such use is Generally Recognized As Safe (GRAS) by scientific procedures.

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Date

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10. REFERENCES

ATCC (2005) *Xanthomonas campestris* (Pammel) Dowson. ATCC The Global Bioresouce Center. <http://www.atcc.org/common/catalog/numSearch/numResults.cfm?at...>. (site visited on September 8, 2005)

Becker, A., Katzen, F., Puhler, A. and Ielpi, L. (1998) Xanthan gum biosynthesis and application: a biochemical/genetic perspective. *Applied Microbiology and Biotechnology* 50:145-152.

Behall, K. M., Scholfield, D. J., van der Sluijs, A. M. C. and Hallfrisch, J. (1998) Breath hydrogen and methane expiration in men and women after oat extract consumption. *Journal of Nutrition* 128:79-84.

Booth, A. N., Hendrickson, A. P. and DeEds, F. (1963) Physiologic effects of three microbial polysaccharides on rats. *Toxicology and Applied Pharmacology* 5:478-484.

Borzelleca, J. F. (1992) Macronutrient substitutes: safety evaluation. *Regulatory Toxicology and Pharmacology* 16:253-264.

Brody, T. (1999a) Regulation of energy metabolism. In *Nutritional Biochemistry*. 2nd Edition. (T. Brody, Ed.). Academic Press, San Diego, CA. p. 157-160.

Brody, T. (1999b) The Krebs cycle. In *Nutritional Biochemistry*. 2nd Edition. (T. Brody, Ed.). Academic Press, San Diego, CA. p. 228-235.

Burdock, G. A. (1997) Xanthan gum. In *Encyclopedia of Food and Color Additives*. Vol. III. CRC Press, Boca Raton, FL. p. 2961-2965.

Cadmus, M. C., Rogovin, S. P., Burton, K. A., Pittsley, J. E., Knutson, C. A. and Jeanes, A. (1976) Colonial variation in *Xanthomonas campestris* NRRL B-1459 and characterization of the polysaccharide from a variant strain. *Canadian Journal of Microbiology* 22:942-948.

Cameron-Smith, D., Collier, G. R. and O'dea, K. (1994) Effect of soluble dietary fibre on the viscosity of gastrointestinal contents and the acute glycaemic response in the rat. *The British Journal of Nutrition* 71:563-571.

CDER (2004) Inactive ingredient search for approved drug products. Xanthan gum. U. S. Food and Drug Administration-Center for Drug Evaluation and Research. <www.acessdata.fda.gov/scripts/cder/iig/getiigWEB.cfm>. (site visited on April 14, 2005)

Clegg, S. M. (1996) Xanthan gum. In *Handbook of Fat Replacers*. (S. Roller and S. A. Jones, Eds.). p. 204-212.

CP Kelco (2006a) RPXG appearance. (Personal Communication)

CP Kelco (2006b) RPXG brine test. (Personal Communication)

CP Kelco (2006c) RPXG specifications. (Personal Communication)

CP Kelco (2006d) Unique characteristics of RPXG. (Personal Communication)

CP Kelco (2006e) Stability of RPXG. (Personal Communication)

Davidson, I. V. (1978) Production of polysaccharide by *Xanthomonas campestris* in continuous culture. *FEBS Letters* 347-349.

Derelanko, M. J. and Hollinger, M. A. (2006) Reproductive toxicology. In *CRC Handbook of Toxicology*. 2nd Edition. CRC Press, Boca Raton, FL. p. 479-489.

Eastwood, M. A., Brydon, W. G. and Anderson, D. M. (1987) The dietary effects of xanthan gum in man. *Food Additives and Contaminants* 4:17-26.

FCC (2003) Xanthan gum. In *Food Chemicals Codex*. 5th Edition. National Academy Press, Washington, DC. p. 504-505.

FDA (1992) Statement of Policy: foods derived from new plant varieties. *Federal Register* 22984-23005.

FDA (2005) Xanthan gum. <http://www.accessdata.fda.gov/scripts/ cdrh/cfdocs/cfcfr/ CFR Search.cfm?fr=172.695 >.

Graham Solomons, T. W. (1984) Molecules with more than one chiral carbon. In *Organic Chemistry*. 3rd Edition. (T. W. Graham Solomons, Ed.). John Wiley & Sons, New York. p. 321.

Hallfrisch, J. and Behall, K. M. (1999) Breath hydrogen and methane responses of men and women to breads made with white flour or whole wheat flours of different particle sizes. *Journal of the American College of Nutrition* 18:296-302.

Hassler, R. A. and Doherty, D. H. (1990) Genetic engineering of polysaccharide structure: production of variants of xanthan gum in *Xanthomonas campestris*. *Biotechnology Progress* 6:182-187.

Health Canada (1994) Guidelines for the safety assessment of novel foods. Volume I. Preamble and guidance scheme for notification. Food Directorate. Health Protection Branch. Health Canada.

Hendrickson, A. P. and Booth, A. N. (1965) Supplementary acute toxicological studies of polysaccharide B-1459. Western Regional Research Laboratory, Albany, CA. Report Number: FDA-FOI No. 05-10897, 2006. p. 119-122.

Ielpi, L., Couso, R. O. and Dankert, M. A. (1981) Xanthan gum biosynthesis. Pyruvic acid acetal residues are transferred from phosphoenolpyruvate to the pentasaccharide-P-P-lipid. *Biochemical and Biophysical Research Communications* 102:1400-1408.

Ikegami, S., Tsuchihashi, F., Harada, H., Tsuchihashi, N., Nishide, E. and Innami, S. (1990) Effect of viscous indigestible polysaccharides on pancreatic-biliary secretion and digestive organs in rats. *The Journal of Nutrition* 120:353-360.

Illiev, I. and Ivanova, I. (2002) Activity profiles of primary metabolism enzymes in *Xanthomonas campestris* strains during xanthan production. *Biotechnology and Biotechnology Equipment* 16:77-82.

IoM and NRC (2004) Methods for predicting and assessing unintended effects on human health. In *Safety of Genetically Engineered Foods: Approaches to Assessing Unintended Health Effects*. National Academies Press, Washington, DC. p. 127-173.

Jana, A. K. and Ghosh, P. (1999) Effect of citric acid on the biosynthesis and composition of xanthan. *Journal of General and Applied Microbiology* 45:115-120.

Jansson, P. E., Kenne, L. and Lindberg, B. (1975) Structure of extracellular polysaccharide from *Xanthomonas campestris*. *Carbohydrate Research* 45:275-282.

Jarman, T. R. and Pace, G. W. (1985) Production of xanthan having a low pyruvate content. Kelco Biospecialities Limited, UK.

JECFA (1987) Toxicological evaluation of certain food additives and contaminants. Xanthan gum. World Health Organization, Geneva, Switzerland.

JECFA (1991) Strategies for assessing the safety of foods produced by biotechnology. World Health Organization (WHO), Geneva, Switzerland.

Kelco Company (1975) *Xanthan gum brochure*. 2nd revised Edition. Kelco Company, San Diego, CA. p. 1-36.

Kibbe, A. H. (2000) Xanthan gum. In *Handbook of Pharmaceutical Excipients*. 3rd Edition. American Pharmaceutical Association, Washington, DC. p. 599-601.

Melton, L. D., Mindt, L., Rees, D. A. and Sanderson, G. R. (1976) Covalent structure of the extracellular polysaccharide from *Xanthomonas campestris*: evidence from partial hydrolysis studies. *Carbohydrate Research* 46:245-257.

OECD (1993) Safety evaluation of foods derived by modern biotechnology. Concepts and principles. Organisation for Economic and Co-Operation Development (OECD), Paris, France. p. 1-74.

Osilesi, O., Trout, D. L., Glover, E. E., Harper, S. M., Koh, E. T., Behall, K. M., O'dorisio, T. M. and Tartt, J. (1985) Use of xanthan gum in dietary management of diabetes mellitus. *American Journal of Clinical Nutrition* 42:597-603.

Ou, S., Kwok, K., Li, Y. and Fu, L. (2001) *In vitro* study of possible role of dietary fiber in lowering postprandial serum glucose. *Journal of Agricultural and Food Chemistry* 49:1026-1029.

PAFA (2006) Food additive safety profile (FASP). Xanthan gum. Priority-Based Assessment of Food Additives (PAFA). Center for Food Safety and Applied Nutrition. US Food and Drug Administration, Washington, DC.

Peters, H. U., Suh, I. S., Schumpe, A. and Deckwer, W. D. (1993) The pyruvate content of xanthan polysaccharide produced under oxygen limitation. *Biotechnology Letters* 15:565-566.

Robbins, D. J., Moulton, J. E. and Booth, A. N. (1964) Subacute toxicity study of a microbial polysaccharide fed to dogs. *Food and Cosmetics Toxicology* 2:545-550.

Sandford, P. A., Pittslby, J. E., Knutson, C. A., Watson, P. R., Cadmus, M. C. and Jeanes, A. (1977) Variation in *Xanthomonas campestris* NRRL B-1459: characterization of xanthan products of differing pyruvic acid content. *American Chemical Society Symposium Series* 45:192-210.

Sandford, P. A., Watson, P. R. and Knutson, C. A. (1978) Separation of xanthan gums of differing pyruvate content by fractional precipitation with alcohol. *Carbohydrate Research* 63:253-256.

Sargent, E. V., Adolph, J., Clemmons, M. K., Kirk, G. D., Pena, B. M. and Fedoruk, M. J. (1990) Evaluation of flu-like symptoms in workers handling xanthan gum powder. *Journal of Occupational Medicine. : Official Publication of the Industrial Medical Association.* 32:625-630.

Shatwell, K. P., Sutherland, I. W. and Ross-murphy, S. B. (1990) Influence of acetyl and pyruvate substituents on the solution properties of xanthan polysaccharide. *International Journal of Biological Macromolecules* 12:71-78.

Silman, R. W. and Rogovin, P. (1972) Continuous fermentation to produce xanthan biopolymer: effect of dilution rate. *Biotechnology and Bioengineering* 14:23-31.

Slodki, M. E. and Cadmus, M. C. (1978) Production of microbial polysaccharides. *Advances in Applied Microbiology* 23:19-54.

Sloneker, J. H. and Orentas, D. G. (1962) Pyruvic acid, a unique component of an exocellular bacterial polysaccharide. *Nature* 194:478-479.

Stankowski, J. D., Mueller, B. E. and Zeller, S. G. (1993) Location of a second *O*-acetyl group in xanthan gum by the reductive-cleavage method. *Carbohydrate research.* 241:321-326.

Sunvold, G. D., Fahey, G. C., Merchen, N. R., Titgemeyer, E. C., Bourquin, L. D., Bauer, L. L. and Reinhart, G. A. (1995) Dietary fiber for dogs: IV. *In vitro* fermentation of selected fiber sources by dog fecal inoculum and *in vivo* digestion and metabolism of fiber-supplemented diets. *Journal of Animal Science* 73:1099-1109.

Sutherland, I. W. (1987) Xanthan lyases - novel enzymes found in various bacterial species. *Journal of General Microbiology* 133:3129-3134.

Tait, M. I., Sutherland, I. W. and Clarke-Sturman, A. J. (1986) Effect of growth conditions on the production, composition and viscosity of *Xanthomonas campestris* exopolysaccharide. *Journal of General Microbiology* 132:1483-1492.

Trout, D. L., Ryan, R. O. and Bickard, M. C. (1983) The amount and distribution of water, dry matter, and sugars in the digestive tract of rats fed xanthan gum. *Proceedings of the Society for Experimental Biology and Medicine* 172:340-345.

USP (2005) Xanthan gum. In *The United States Pharmacopeia (USP 29): The National Formulary (NF 24)*. United States Pharmacopeial Convention, Rockville, MD. p. 3459-3460.

Woodard, G., Woodard, M. W., Mcneely, W. H., Kovacs, P. and Cronin, M. T. (1973) Xanthan gum: safety evaluation by two-year feeding studies in rats and dogs and a three-generation reproduction study in rats. *Toxicology and Applied Pharmacology* 24:30-36.

11. APPENDIX I

Title 21. Code of Federal Regulations. 172.695. Xanthan Gum

The food additive xanthan gum may be safely used in food in accordance with the following prescribed conditions: (a) The additive is a polysaccharide gum derived from *Xanthomonas campestris* by a pure-culture fermentation process and purified by recovery with isopropyl alcohol. It contains D-glucose, D-mannose, and D-glucuronic acid as the dominant hexose units and is manufactured as the sodium, potassium, or calcium salt. (b) The strain of *Xanthomonas campestris* is nonpathogenic and nontoxic in man or other animals. (c) The additive is produced by a process that renders it free of viable cells of *Xanthomonas campestris*. (d) The additive meets the following specifications:

(1) Residual isopropyl alcohol not to exceed 750 parts *per* million.

(2) An aqueous solution containing 1 *percent* of the additive and 1 *percent* of potassium chloride stirred for 2 hours has a minimum viscosity of 600 centipoises at 75°F, as determined by Brookfield Viscometer, Model LVF (or equivalent), using a No. 3 spindle at 60 rpm, and the ratio of viscosities at 75°F and 150°F is in the range of 1.02 to 1.45.

(3) Positive for xanthan gum when subjected to the following procedure:

Locust Bean Gum Gel Test

Blend on a weighing paper or in a weighing pan 1.0 gram of powdered locust bean gum with 1.0 gram of the powdered polysaccharide to be tested. Add the blend slowly (approximately \1/2\ minute) at the point of maximum agitation to a stirred solution of 200 milliliters of distilled water previously heated to 80°C in a 400-milliliter beaker. Continue mechanical stirring until the mixture is in solution, but stir for a minimum time of 30 minutes. Do not allow the water temperature to drop below 60°C. Set the beaker and its contents aside to cool in the absence of agitation. Allow a minimum time of 2 hours for cooling. Examine the cooled beaker contents for a firm rubbery gel formation after the temperature drops below 40°C.

In the event that a gel is obtained, make up a 1 *percent* solution of the polysaccharide to be tested in 200 milliliters of distilled water previously heated to 80°C (omit the locust bean gum). Allow the solution to cool without agitation as before. Formation of a gel on cooling indicates that the sample is a gelling polysaccharide and not xanthan gum.

Record the sample as ``positive" for xanthan gum if a firm, rubbery gel forms in the presence of locust bean gum but not in its absence. Record the sample as ``negative" for xanthan gum if no gel forms or if a soft or brittle gel forms both

with locust bean gum and in a 1 *percent* solution of the sample (containing no locust bean gum).

(e) The additive is used or intended for use in accordance with good manufacturing practice as a stabilizer, emulsifier, thickener, suspending agent, bodying agent, or foam enhancer in foods for which standards of identity established under section 401 of the Act do not preclude such use.

(f) To assure safe use of the additive:

(1) The label of its container shall bear, in addition to other information required by the Act, the name of the additive and the designation ``food grade".

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

(4) Positive for xanthan gum when subjected to the following procedure:

Pyruvic Acid Test

Pipet 10 milliliters of an 0.6 *percent* solution of the polysaccharide in distilled water (60 milligrams of water-soluble gum) into a 50-milliliter flask equipped with a standard taper glass joint. Pipet in 20 milliliters of 1N hydrochloric acid. Weigh the flask. Reflux the mixture for 3 hours. Take precautions to avoid loss of vapor during the refluxing. Cool the solution to room temperature. Add distilled water to make up any weight loss from the flask contents.

Pipet 1 milliliter of a 2,4-dinitro phenyl hydrazine reagent (0.5 *percent* in 2N hydrochloric acid) into a 30-milliliter separatory funnel followed by a 2-milliliter aliquot (4 milligrams of water-soluble gum) of the polysaccharide hydrolyzate. Mix and allow the reaction mixture to stand at room temperature for five minutes. Extract the mixture with 5 milliliters of ethyl acetate. Discard the aqueous layer.

Extract the hydrazone from the ethyl acetate with three 5 milliliter portions of ten *percent* sodium carbonate solution. Dilute the combined sodium carbonate extracts to 100 milliliters with additional ten *percent* sodium carbonate in a 10-milliliter volumetric flask. Measure the optical density of the sodium carbonate solution at 375 millimicrons.

Compare the results with a curve of the optical density *versus* concentration of an authentic sample of pyruvic acid that has been run through the procedure starting with the preparation of the hydrazone. Record the *percent* by weight of pyruvic acid in the test polysaccharide. Note ``positive" for xanthan gum if the sample contains more than 1.5 *percent* of pyruvic acid and ``negative" for xanthan gum if the sample contains less than 1.5 *percent* of pyruvic acid by weight.

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