Vol 3 Tab 26]. This survey represents data that has been subjected to statistical analysis. The CSFII food categories are shown in Table 28. The intakes presented in the table represent the mean consumption of adult men and women 20 years and over.

For example, food sub-categories 01A through 01C in Appendix IV (the NAS categories) are averaged to yield an average usage level for Yeast Bread and Rolls on Table 28 (the CSFII categories). Since the CSFII data has withstood statistical analysis, percentage use levels for Xanthan gum are multiplied by the mean intakes indicated in the CSFII database. The resulting total per food category is then summed to yield a total mean daily intake. Please note that zeros have been entered for Xanthan for certain food categories where there is unlikely to be significant usage, e.g., fresh vegetables and nuts. The total mean intake from Table 28 may then be doubled to estimate the 90th percentile (in accordance with the guidance document).

III. Probable Consumption, (continued)

In the absence of data to the contrary, Tables 28 and 29 must be viewed as the total use levels of both types of xanthan, since except for the residual alcohol levels they are totally interchangeable.

Table 28 includes one additional category of use not encountered in CSFII (1996). This is the potential intake of Xanthan gum as a dietary supplement taking advantage of one of the physiological effects discussed in subsection D above. The lower residual alcohol levels in Xanthan gum purified by recovery with ethanol could accelerate the realization of this potential use of Xanthan gum in the human diet.

The range of potential intake levels of Xanthan gum as a dietary supplement presented in Table 28 is 1.5 to 4.5 grams/day and has been estimated as follows.

III. Probable Consumption, (continued)

Table 27

Maximum Use of Xanthan Gum Purified by Recovery with Ethanol In the Food Categories Listed by the US FDA At 21 CFR §170.3 (n)

Food Category Code	GRAS Food Additive Categories	Realistic Levels of Xanthan Gum (%)
01	Baked Goods, Baking Mixes	0.2
02	Beverages alcoholic	0
03	Beverages and beverage bases, non-alcoholic	0.05
04	Breakfast Cereals	0
05	Cheeses	0.2
06	Chewing Gum	0
07	Coffee and tea**	0.05
08	Condiments and relishes	0.3
09	Confections and frostings	0.2
10	Dairy product analogs	0.1
11	Egg Products	0
12	Fats and oils	0.3
13	Fish Products	0.1
14	Fresh eggs	0
15	Fresh fish	0
16	Fresh fruits and fruit juices	0
17	Fresh Meats	0
18	Fresh poultry	0
19	Fresh vegetables	0
20	Frozen dairy desserts and mixes	0.2
21	Fruit and water ices	0.2
22	Gelatins, puddings and fillings	0.1
23	Grain products and pastas	0
24	Gravies and Sauces	0.15
25	Hard candy	0
26	Herbs, seeds, spices, seasonings, blends	0

Table 27, (continued)

Food Category Code	GRAS Food Additive Categories	Realistic Levels of Xanthan Gum (%)
27	Jams and jellies, home prepared	0
28	Jams and jellies commercial	0.3
29	Meat products	0.4
30	Milk, whole and skim	0
31	Milk products	0.25
32	Nuts and Nut products	0
33	Plant protein products	0.1
34	Poultry products	0
35	Processed fruits and fruit juices	0.2
36	Processed vegetables and vegetable juices	0.2
37	Snack foods	0.05
38	Soft Candy	0.1
39	Soups, home prepared	0
40	Soups and soup mixes	0.15
41	Sugar, white granulated	0
42	Sugar substitutes	0
43	Sweet Sauces, toppings, and syrups	0.5

Table 28
Estimated Intake of Xanthan Gum in Selected Food Categories

Major Food Codes	Food Category	Xanthan Use (%)	Food Intake (g/day)	Xanthan Intake (g/day)	CSFII
	Baked goods, baking mixes				
51	Yeast Breads and Rolls	0.05	50	0.025	1996
52, 55	Quick breads, pancakes, etc.	0.2	0.1	0.000002	1996
53	*Cakes, cookies, pastries, pies	0.2	38	0.076	1996
58	Mixtures mainly grain	0.05	107	0.0535	1996
	Breakfast cereals				
571 - 574, 578	Ready-to-eat cereals	0	17	0	1996
	Grain products and pastas				
562	Rice	0	19	0	1996
561	Pasta	0	21	0	1996
54	Snack foods(crackers, chips)	0.05	12	0.006	1996
	Total Vegetables		132		1996
	Fresh vegetables	0	0	0	1996
	Processed vegetables, juices	0.05	66	0.033	1996
	Total Fruits		162		1996
612	Citrus juices	0.075	59	0.044	1996
621	Dried fruits	0	0	0	1996
641,642	Non-citrus juices and nectars	0.05	26	0.013	1996
	Fruits and mixtures		0		1996
	Milk products				
114	Yogurt	0.2	8	0.016	1996
131,132, 133, 134	Milk desserts	0.1	24	0.024	1996
140-147	Cheese	0.1	16	0.016	1996
116, 118	Milk-Based Beverages	0.2	34	0.068	1966
	Meat Products				
252	Sausages, processed meats	0.2	21	0.042	1996
271,272,273,274	Mixtures mainly meat	0.1	99	0.099	1996

Table 28, (continued)

Major Food Codes	Food Category	Xanthan Use (%)	Food Intake (g/day)	Xanthan Intake (g/day)	CSFII
	Eggs				
321,323,324, 33,34	Egg Products	0	9**	0	1996
	Legumes				
	Legumes	0.1	28	0.028	1996
	Nuts and Nut Products				
	Nuts and Nut Products	0	0	0	1996
	Fats and Oils				
832	Salad dressings	0.3	8	0.024	1996
	Total sugars and sweets				
917,918	Candy (Soft and Hard)	0.1	7	0.007	1996
911 - 916	Other Sugar Products***	0	15	0	1996
	Beverages Non-Alcoholic				
	Coffee	0	254	0	1996
923	Teas	0.05	128	0.064	1996
925	Low Cal. Fruit drinks and ades	0.05	18	0.009	1996
92400100	Low Cal. Carbonated drinks	0	74	0	1996
	Total Intake (grams)			**0.648**	
	Potential Dietary Supplement Intake				**1.5-4.5**
	* Includes dietetic bars				
	** Includes 50% of egg intake for processed eggs				
	*** Includes Jams, Jellies, etc.				

Using CFSII intake data, the maximum estimated mean intake for Xanthan gum was 0.65 grams per day. An estimated 90th percentile may be calculated by multiplying 0.65 X 2. The value generated is thus, 1.3 grams per day. This does not include any potential intake for Xanthan gum as a supplemental source of soluble dietary fiber, or as an ingredient in special uses. Intakes for the technical effect of a nutritional supplement are estimated to range from 1.5 – 4.5 grams per day. This range is consistent with labeling recommendations

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in the dietary supplement industry, and has been previously reported to FDA in GRAS Notice 000047 for arabinogalactan, also a polysaccharide [US FDA, GRN 000047 Vol 4 Tab 73]. Additionally, an intake of Xanthan gum of 15 times the 10mg/kg b.w. (0.6 grams per day for a 60 kg human) previously approved by the Joint FAOWHO Expert Committee on Food Additives were fed to healthy human volunteers, and no effects of toxicological significance were reported [Eastwood, 1987 Vol 2 Tab 23]. At intake levels ranging from 10.4 to 12.9 grams per day, the investigator recorded effects typical for dietary fibers; i.e., fecal bulking and altered intestinal transit times [Eastwood, 1987 Vol 2 Tab 23 and Institute of Medicine Report, 2001 Vol 3 Tab 31].

For the food products listed above, all usage levels for Xanthan gum fell between 0.05% and 0.3% of the finished food product. The highest levels of potential intake were found in the milk products area (0.124 grams per day), which encompasses milk desserts, milk-based beverages, and cheese; processed meat products (0.141 grams per day); and baked goods (0.155 grams per day).

A number of conservative assumptions are inherent in the guidance document provided by FDA for estimating exposure to direct food additives. These are:

3. That a direct additive will be used in the broadest possible food groups that can be considered appropriate for the ingredient;
4. That each food in a given food category will contain the concentration of Xanthan gum presented;
5. That the direct additive will be contained in all foods within a category.

The Notifiers submit that consumption of Xanthan gum purified by recovery with ethanol will not reach the levels suggested by the conservative assumptions shown above. Several logical reasons can be suggested for this assumption:

1. Manufacturers of Xanthan gum purified by recovery with ethanol are limited, and currently, there are none in the United States. Data has been presented, which shows that the world's supply of food grade Xanthan is approximately 22,500 metric tonnes per year (47,250,000 lbs. per year). Additionally, the majority of the supply is recovered with isopropanol. If the total production were divided among the US population (129,452 lbs. per day ÷ 250,000,000 people) the daily intake would be 0.0005 lbs. per day or 0.235 grams per person per day. In which case, the 90th percentile would equal 0.470 grams per day /person.
2. Another ameliorating factor is the fact that Xanthan gum purified by recovery with ethanol will compete with other hydrocolloids, which may have similar functions in foods (See Table 23 above), as well as with Xanthan purified by recovery with ethanol. Therefore, consumption of this specific product will be limited across the total US population, with the highest consumption most likely being attributed to brand loyal consumers.
3. Xanthan gum purified by recovery with ethanol is not expected to have technical advantages that would result in its use in all foods within a given category. Many of the functional applications are similar or marginally advantageous to other more common, less costly ingredients. Table 29 below illustrates the market penetration of currently available Xanthan. Note that market penetration ranges from minimal to >90% for salad dressings and whipped toppings. Even though some food processors find it advantageous to use Xanthan gum in a particular food product does not mean all processors hold to this view. This is why the market penetration figures given in Table 29 are less than 100%.

Given these factors, the Notifiers feel it is reasonable to assume that the daily exposure level for Xanthan gum purified by recovery with ethanol would be substantially less than the average mean intake estimate of 0.65 g/person/day, which resulted from the calculation in Table 28. The Notifiers submit that there is a sufficient margin of safety to support

this very conservative maximum consumption level. All feeding studies conducted on humans and various species of animals have demonstrated that Xanthan gum is safe, even when consumed at relatively high levels [Eastwood, 1993 Vol 2 Tab 22 and Woodard, *et al.*, 1973 Vol 4 Tab 81]. The supporting safety data are discussed in detail in Section IV, Summary for the Determination the Xanthan Gum is GRAS.

Table 29
Estimated Market Penetration
For Xanthan Gum Purified by Recovery with Ethanol

Food Type	*Commercial Use Level (%)*	Estimated Market Penetration(% of Products)
Milk Products		
Whipped cream	**0.15**	Minimal
Whipped topping	**0.2**	>90
Light custards	**0.1**	20
Flavored milk	**0.2**	Minimal
Frozen yogurt	**0.2**	Minimal
Sherbet	**0.2**	Minimal
Mousses	**0.25**	Minimal
Ice cream	**0.1**	<10
Cottage cheese	**0.1**	30
Cream cheese	**0.2**	90
Dry Mixes (As eaten)		
Milk shakes	0.15	10
Citrus and fruit flavored drinks	0.5	25
Soups	0.15	25
Salad dressings	0.5	50
Cakes and muffins	0.2	25
Instant desserts	0.3	20
Flavored coffee	0.2	20

Table 29, (continued)

Meat and Fish Products		
Surimi	0.1	Minimal
Processed meats	0.4	Minimal
Ices, Water-Ices		
Sorbets	0.1	<10
Beverages		
Fruit drinks	0.05	**80**
Bottled coffees and teas	0.05	**Minimal**
Salad Dressings		
High oil:50-60%	0.2	**>90**
Medium oil: ~30%	0.25	**>90**
Low oil: 10-20%	0.3	**>90**
No oil	0.5	**>90**
Sauces and Soups		
Tomato ketchup	0.4	**Minimal**
Pizza sauce	0.4	**Minimal**
Barbeque sauce Marinades	0.5	**40**
Meat sauces	0.5	**40**
Cream soup	0.15	**15**
Dips, salsas	0.2	**15**
Chocolate syrup	0.15	**15**
	0.4	**90**
Fruit and Vegetable Preparations		
Dietetic jams	0.3	**Minimal**
Pickles, relishes and chutney	0.3	**Minimal**
Jelly Toppings	0.3	**Minimal**
Frozen Foods		
Cakes	0.1	**50**
Complete meals	0.3	**15**
Retorted pouches	0.15	**50**

F. Current Good Manufacturing Practice Use Levels

Table 30 below provides a summary of current Good Manufacturing use levels for Xanthan gum in major food categories. Use levels typically range from 0.05% for fruit drinks to 0.2% for milk-based desserts [Kelco, Product Literature, undated Vol 3 Tab 36 and Urlacher, *et al.*, 1997 Vol 4 Tab 71].

Table 30
Current Good Manufacturing Practice Use Levels in Foods for Xanthan Gum Purified by Recovery with Ethanol

Food Category[1]	Use Level[2]
Baked Goods	0.2%
Rice and Other Grain-Based Dishes	0
Vegetables and Vegetable Dishes	0.2%
Fruits and Fruit Preparations	0.05%
Milk Desserts/Cheese Products	0.2%
Meat, Poultry and Fish	0.2%
Eggs and Egg Dishes	0
Legumes and Legume Dishes	0.1%
Sugars and Sweets	0.1%
Coffee	0.05%[3]
Fruit Drinks and Ades	0.05%

1. Major food categories are based on those used in USDA's *Continuing* Survey of Food Intake by Individuals with rice and other grain-based dishes shown as a separate category [Enns, *et al.*, 1997 Vol 3 Tab 26].
2. Maximum use levels are those which have been formulated into test applications, or have been prepared for commercial test markets.
3. Xanthan gum is currently found only in certain flavored bottled coffees.

G. Other Foods Prepared with Ethanol

Many consumer food products (other than alcoholic beverages) are commercially prepared using ethanol either as an ingredient, a carrier, or an extractant. In this regard ethanol has been consumed as part of the normal diet for many decades. Xanthan gum purified by recovery with ethanol, therefore, does not present a novel process to the food manufacturing industry. The following citations are used to demonstrate the variety of foods prepared using ethanol. In each case a certain amount of residual ethanol (ranging from parts per million to several percent) can be expected to remain in the final food product, either intentionally, or as a result of processing.

1. Ethanol As an Extractant

Santamaria, *et al.,* publishing in the *Journal of Agriculture and Food Chemistry* studied the selective extraction of capsaicinoids and carotenoids from chili guajillo *(Capsicum annum L.)* "puya" flour. When ethanol was used as solvent, 80% of capsaicinoids and 73% of carotenoids were extracted, representing an interesting alternative for the substitution of hexane in industrial processes. Additionally, when the flour was pretreated with enzymes to break the cell wall and then dried, extraction in ethanol increased to I % and 7% for carotenoid and capsaicinoid, respectively. A selective two-stage extraction process after the treatment with enzymes was proposed by the authors. The first step used 300% (v/v) ethanol and released up to 60% of the initial capsaicinoids, and the second extraction step with industrial ethanol permited the recovery of 93% of the carotenoids present in the flour [Santamaria, *et al.,* 2000 Vol 4 Tab 58].

The objective of work by Regitano-d'Arce, *et al.,* was to study and identify the necessary processing steps for obtaining high quality sunflower seed protein concentrate and isolate using ethanol as the extractant for the oil. The researcher's work was part of a project to develop a renewable solvent system for sunflower

seed oil recovery and possible downstream processing of the meal. Both 99% and 90% ethanol were employed in the extractions to produce the concentrate [Regitano-d'Arce, *et al.,* 1994 Vol 4 Tab 55].

In a second study, the investigators determined the functional properties of sunflower seed meal var. *Anhandy* obtained through intermittent ethanol oil extraction using four concentrations (99%, 96%, 93% and 90%). Meal nitrogen solubility and dispersibility, and oil absorption capacities were evaluated. The highest protein solubility (70%) was obtained using 93% extraction meal. 99% and 90% extracted meals showed the best water absorption performances (11.4 ml H_2O/g protein), while 96% meal had the highest oil absorption (7.3 ml free oil absorbed/g protein). The highest nitrogen dispersibility was found in 96% and 99% meals (1.6% dispersed nitrogen or approximately 27% yield). Nitrogen solubility assays in salt solutions indicated that pH 11 yielded optimum results; however, the yield was lower than in aqueous solutions. The authors concluded that meals obtained with more concentrated ethanol-water solutions were needed for further processing into concentrates and isolates [Regitano-d'Arce, *et al.,* 1994 Vol 4 Tab 55].

Tate *et al.*, publishing in *Plant Foods and Human Nutrition* characterized a commercial peanut meal produced by expeller pressing of peanuts, which had been extracted with n-hexane, and 80% ethanol. The extraction was followed by sieving through an 80 mesh screen, to remove residual oil, pigments, bitter flavors, and fibrous material. The processed meal exhibited comparable composition to defatted peanut flour prepared in the laboratory by solvent extraction of full-fat peanut meal. However, the authors reported that the processed meal exhibited a low methionine content and *in vitro* protein digestibility as compared with traditionally defatted peanut flour. The study indicated that the processed meal could be blended with wheat flour to a level of 10% (w/w) to prepare acceptable cookies with improved protein and mineral content [Teta *et al.*, 1990 Vol 4 Tab 68].

A Turkish study described the extraction of lycopene and beta-carotene from tomato paste waste using supercritical carbon dioxide (SC-CO_2). To optimize supercritical fluid extraction (SFE) of these components, a factorially designed experiment was conducted. The authors assessed several factors including: temperature of the extractor (35, 45, 55, and 65° C), the pressure of the extraction fluid (200, 250, and 300 bar), addition of cosolvent (5, 10, and 15% ethanol), extraction time (1, 2, and 3 h), and CO_2 flow rate (2, 4, and 8 kg/h). The total amounts of lycopene and beta-carotene in the tomato paste waste, extracts, and residues were determined by HPLC. A maximum of 53.93% of lycopene was extracted by SC-CO_2 in 2 h (CO_2 flow rate = 4 kg/h) at 55° C and 300 bar, with the addition of 5% ethanol as a cosolvent. Half of the total beta-carotene was extracted in 2 h (flow rate = 4 kg/h), at 65° C and 300 bar, with the addition of 5% ethanol [Baysal *et al.*, 2000 Vol 2 Tab 4].

Many herbal preparations are extracted and/or stored in ethanol. A study published in *Biofactors* described *Curcuma longa* extracts. The study evaluated the antioxidant capacity of a *Curcuma longa* extract on the lipid peroxidation of liver mitochondria and microsome membranes in atherosclerotic rabbits. Male rabbits fed a 3% (w/w) lard and 1.3% (w/w) cholesterol diet were randomly assigned to three groups. Two groups were treated with different dosages of a turmeric extract (A and B) and the third group (control) with a curcumin-free solution. Following chemical induction of damaging oxidants productions in the liver, the mitochondria and microsomes were analyzed. Group A, which was given the extract, had the lowest concentration of mitochondrial hydroperoxides. In microsomes, the basal hydroperoxide levels were found to be similar in all groups but, after the induction of oxidation, the control group registered the highest value. The same trend was seen in mitochondria. These findings suggested that active compounds in *Curcuma sp.* extract may be protective in preventing lipoperoxidation of subcellular membranes in a dosage-dependent manner [Ramirez-Tortosa, *et al.*, 1999 Vol 4 Tab 54 and Quiles, *et al.*, 1998 Vol 4 Tab 53].

2. *Ethanol as a Supplement to Fermentation*

Chiou *et al.*, published a study in 1999 in which the research team investigated the flavor enhancing effects of ethanol enriched-miso fermentations. Steam-cooked soybeans and rice koji were combined (1:1, w/w), mixed with 5% (w/w) NaCl and ground into a fine paste. Samples (30 g) were deposited in nylon/polyethylene plastic bags and supplemented with 10 ml of aqueous ethanol solutions to give concentrations of 0, 2.5, 5, 7.5, 10, 15, 20, and 25% ethanol. Mixtures were homogenized, sealed, and incubated at 28° C for eight weeks [Chiou *et al.*, 1999 Vol 2 Tab 14].

The authors found that the addition of ethanol effected the microbial profile of the resulting product. Mold populations were less than 3 log10 CFU/g in all miso products after four weeks of fermentation. Yeast populations increased to 6.1 log10 CFU/g in the control (0% added ethanol) during the first week of fermentation and remained stable throughout the eight-week fermentation period. Interestingly, no yeasts were detected in products containing 5-25% ethanol. Populations of lactic acid bacteria (LAB) increased to 6 log10 CFU/g after one week of fermentation in products containing 0 and 2.5% ethanol. However, after eight weeks of fermentation, LAB populations in all products dropped to less than 4 log10 CFU/g. Rapid decreases in pH were found to occur only in products supplemented with 0 or 2.5% ethanol. Analysis of soluble protein in miso products containing various ethanol concentrations during the eight-week fermentation period revealed that protease activity remained active in products supplemented with less than 10% ethanol. Total soluble carbohydrate and glucose contents were higher in products supplemented with 5, 7.5 and 10% ethanol than in other products [Chiou *et al.*,1999 Vol 2 Tab 14].

However, discoloration (browning) during fermentation occurred most rapidly in products supplemented with 5 or 7.5% ethanol. Sensory evaluation of the low-salt (5%) product supplemented with 7.5% ethanol during fermentation showed that those products had normal or enhanced flavor ratings in comparison to a commercial product [Chiou *et al.*,1999 Vol 2 Tab 14].

Ku, *et al.*, evaluated small-scale volatile extraction from ethanol-supplemented low-salt miso and characterized the extracted flavors via GC-MS. An extraction apparatus was equipped with a nitrogen-flushing vessel to purge volatiles from a 10-g miso solution fermented at 40° C. The apparatus was fitted with a reflux condenser to recover water, a coiled cold-trap to separate ethanol, and a glass-lined stainless (GLS) trap filled with Tenax TA for flavor adsorption. Volatiles in the GLS tube were released with a thermal desorption device and condensed with a Micro-cryo trap prior to connection with GC and GC-MS for characterization [Ku, *et al.*, 2000 Vol 3 Tab 37].

The analysis revealed a broad volatile profile comprising 9 categories of functional groups and 97 identified compounds. Because of the ethanol supplementation (7.5%) during the miso fermentation, most volatiles except alcohols and acetals in the low-salt products (5% NaCl) were higher than those in the control products fermented with 9% NaCl and 0% ethanol. The authors concluded that the supplementation of ethanol in miso production not only enabled a low-salt miso fermentation but also enhanced flavor formation [Ku, *et al.*, 2000 Vol 3 Tab 37].

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III. Probable Consumption, (continued)

3. Ethanol in Canned Foods

Ethanol has been used to preserve canned salmon. As a result standardized methods have been developed to measure its concentration. Hollingworth, *et al.* Reported on a six-laboratory collaborative study to develop a headspace gas chromatographic method for determination of ethanol in the aqueous phase of canned salmon. Ethanol is determined by a headspace sampling technique with tert-butanol as the internal standard, using a gas chromatograph equipped with a Super Q column and a flame ionization detector. With outliers excluded, the mean recoveries from samples spiked with 25.1 and 78.4 ppm ethanol were 112 and 110%, respectively. As a result of this work, the method was adopted for official first action [Hollingworth, *et al.* 1986 Vol 3 Tab 29].

McLachlan, *et al.*, published a study in which automated headspace sampling was used in conjunction with analysis by gas chromatography to determine ethanol content in the canned salmon. They noted that the thermal process for the commercial sterilization of canned salmon has been shown to provide an effective extraction of the ethanol so that the fluid removed from the can may be used as the analytical sample with minimal preparation prior to analysis. In their work ethanol content was measured directly, without the need for an internal standard, by either GC/MS or GC/FID. They concluded that the headspace autoanalyzer allowed for a rapid determination of ethanol with greater reproducibility than could be obtained with manual injection systems. Atlantic sea scallop meats containing ethanol were also successfully analyzed by this technique [McLachlan, *et al.*, 1999 Vol 3 Tab 42].

4. Ethanol in Traditional Fermented Foods

Ethanol is present in Mexican pozol, a type of dough product. The dynamics of the microbial community responsible for the traditional fermentation of maize in the production of Mexican pozol was investigated by ben Omar using a complex approach which combined (i) microbial enumerations, (ii) fingerprinting of total community DNA with bacterial and eukaryotic primers and sequencing of partial 16S ribosomal DNA (rDNA) genes, (iii) quantification of rRNAs from dominant microbial taxa by using phylogenetic oligonucleotide probes, and (iv) analysis of sugars and fermentation products. Using these techniques, a *Streptococcus* species was found to dominate the fermentation of the dough, and accounted for between 25 and 75% of the total flora throughout the process. Results also showed that the initial epiphytic aerobic microflora was replaced during the first 2 days by heterofermentative lactic acid bacteria, including a close relative of *Lactobacillus fermentum*, which produced lactic acid and ethanol; this heterolactic flora was then progressively replaced by homofermentative lactic acid bacteria (mainly close relatives of *L. plantarum, L. casei, and L. delbrueckii*) which continued acidification of the maize dough. At the same time, a very diverse community of yeasts and fungi developed, mainly at the periphery of the dough. The results demonstrated that a relatively high number of species, at least six to eight, were needed to perform this traditional lactic acid fermentation. The presence of *Bifidobacterium, Enterococcus*, and *Enterobacteria* suggested a fecal origin of some important pozol microorganisms [ben Omar, *et al.*, 2000 Vol 2 Tab 6].

5. Ethanol in Candy

Ethanol (generally in the form of flavored liqueurs) containing candies have long been a traditional holiday confection in Western Europe. A paper from Croatia describes a simple gas chromatographic method for quantification of ethanol in distillates of chocolate shell pralines and fillings. The samples were prepared in two steps. The first step consisted of ethanol distillation from the product and the second involved capillary gas chromatography of 10% v/v distillate with the expected ethanol content between 0.06% and 2.5% w/w. Quantification was carried out using iso-propanol as internal standard. The range of linear method response was 0.05-3.16% w/w of ethanol, which corresponded to products with ethanol content between 0.5 and 31.6% w/w [Pintaric, 1999 Vol 3 Tab 50].

6. Ethanol in Flavoring

Common household flavoring products, often contain ethanol as a diluent. For example, vanilla flavoring may contain 35% ethanol on a volume basis [McCormick Product Label, 2001 Vol 3 Tab 41]. These flavorings are frequently consumed in unheated foods, such as desserts, in which case a small amount of ethanol would be consumed as a part of the product. No untoward effects have been

7. Ethanol in Purification

Ethanol purification is known in the food processing industry. Ethanol is either used to purify certain sugars (D-ribose) or is co-produced with certain sugar alcohols (xylitol) [US Patent 3,919046 Vol 4 Tab 74 and US Patent 3,970,522 Vol 4 Tab 75]. Ethanol is also approved for the recovery of Xanthan gum in the EU. In 1999 at the 53rd meeting, JECFA approved new specifications for Xanthan gum, which include levels of ethanol at not more than 500 mg/kg [JECFA, 1999 Vol 3 Tab 34].

The examples discussed above illustrate the long history of safe use of ethanol in food processing on a worldwide basis. Many of the foods containing ethanol are traditional, and therefore have been consumed for centuries. Table 31 lists the uses and the countries reporting those uses, which have been cited above.

Table 31
Ethanol in Food Processing

Product	Country	Use/Function
Miso	China	Supplement To Fermentation/Flavoring
Canned Salmon	Canada	Preservation
Capsaicinoids And Carotenoids	Mexico	Extractant
Beta-Carotene And Lycopene	Turkey	Extractant
Pozol Dough	Mexico	Product Of Fermentation
Curcuma Longa	Spain	Extractant
Candy	Croatia	Flavoring
D-Ribose	USA & China	Purification
Xanthan Gum	Western Europe & China	Recovery/Purification

H. Consumption of Ethanol from Xanthan Gum Purified by Recovery with Ethanol

The mean consumption for Xanthan gum purified by recovery with ethanol has been estimated to equal 0.65 grams per day for adult's 20 years and over (Table 31). Ethanol determinations on the product by ISI have shown the maximum ethanol content to be 12 ppm [ISI Raw Data, 2001 Vol 3 Tab 32]. Therefore consumption of residual ethanol in Xanthan gum is likely to be equal to or less than 7.8 micrograms per day (0.0000078 g). Even at the 90th percentile, consumption would remain at the microgram level (0.000016 grams per day). Provided that the ethanol used for Xanthan gum recovery is food grade, this level should pose no threat to human safety, and is indeed less than the ethanolic content of many traditional foods, such as candies.

Section IV

Section IV: Detailed Summary of the Basis for the Determination that Ingredients Solutions, Inc. and ZZBP Gum is GRAS

A. Introduction

The use of Xanthan gum as a food ingredient in human nutrition has been investigated for several decades [Sutherland, 1986 Vol 4 Tab 66]. While previous investigations have focused on the technical effects of Xanthan gum in food formulations, recent research has demonstrated that Xanthan gum functions physiologically in the body as a source of soluble fiber [Ou, *et al.*, 2001 Vol 3 Tab 49 and Eastwood, 1993 Vol 2 Tab 22].

Xanthan gum purified by recovery with ethanol is a multiple-use direct food additive, whose intended uses will encompass those approved for Xanthan gum recovered with isopropanol at 21 CFR 172.695. Over the years food formulators in the edible gum industry have developed intended uses for Xanthan gum which go beyond those currently listed under 21 CFR 172.695. Therefore, the sponsors of this report have expanded the applicable technical effects (from the list of 32 at 21 CFR 170.3 (o)) for Xanthan gum to include those applications that have been developed since the original food additive regulation was published.

In order to be used as an ingredient in foods, Xanthan gum must first be prepared as a solution, and suggested procedures for the preparation of Xanthan solutions have been published [Urlacher *et al.,* 1997 Vol 4 Tab 71, Sworn, 2000 Vol 4 Tab 67, and Povey, 2001 Vol 4 Tab 51].

When properly prepared, Xanthan gum provides high viscosity at rest and high pseudoplasticity, and therefore, has advantages for use in such applications as salad dressings and whipped toppings. Emulsions stabilized with Xanthan gum are not affected by pH levels as low as 3.5, salt concentrations as high as 15%, or thermal treatments, such as UHT pasteurization [Urlacher, *et al.,* 1997 Vol 4 Tab 71 and Morris, 1990 Vol 3 Tab 47].

The concentration of Xanthan gum used in foods, is often dependent upon the amount of oil present. For instance approximately 0.2-0.3% by weight is often used in high oil salad dressing formulations (50-60% oil), while approximately 0.4%-0.6% is typically used in low oil products (10-20% oil) [Morris, 1990 Vol 3 Tab 47 and Urlacher, *et al.,* 1997 Vol 4 Tab 71].

Even at low concentrations, Xanthan gum imparts a high viscosity to sauces and gravies. Xanthan gum is a desirable food ingredient, because its pseudoplasticity is reported to give sauces and gravies clean mouthfeel and good flavor release [Povey, 2001 Vol 4 Tab 51 and Urlacher, *et al.,* 1997 Vol 4 Tab 71].

Xanthan gum may be used alone or in combination with other hydrocolloids in dairy products, where the high yield value and pseudoplasticity of Xanthan produces improved stabilization of the air cells in whipped products. In bakery products Xanthan reduces the loss of water during cooking and storage of the final food product [Morris, 1990 Vol 3 Tab 47, Urlacher, *et al.,* 1997 Vol 4 Tab 71, and Povey, 2001 Vol 4 Tab 51]. In toppings and fillings, Xanthan improves texture, controls syneresis, and helps to improve freeze-thaw stability [Morris, 1990 Vol 3 Tab 47].

B. Detailed Summary

A summary of current Good Manufacturing Practice use levels for Xanthan gum in major food categories showed that use levels typically ranged from 0.05% for fruit drinks to 0.2% for milk-based desserts [Kelco, Product Literature, undated Vol 3 Tab 36 and Urlacher, *et al.,* 1997 Vol 4 Tab 71].

A study published by Industrial Market Research has indicated that approximately 34,409 metric tonnes of Xanthan gum are produced annually worldwide [Industrial Market Research, 1998 Vol 3 Tab 30]. Approximately 65% of this amount, or about 50 million pounds, is sold into the food industry.

The commercial Xanthan gum product currently being produced or sold in the U.S.

Nutritionally, Xanthan gum purified by recovery with ethanol comprises approximately 73.2% carbohydrate, of which nearly 100% is dietary fiber. Protein levels range from 5-6%, and fat content is zero. The fixed acetic acid and pyruvic acid content of the product is 20%, and there are significant quantities of calcium (0.3-0.7%), potassium (0.4-0.6%), and sodium (0.5-0.8%) present. Energy values for the gum are very low, at <0.6 kcal/g [ZZBP Raw data, 2000 Vol 4 Tab 82].

Using CFSII (1996) intake data, the maximum estimated mean intake for Xanthan gum is 0.65 grams per day, and all usage levels for Xanthan gum in a standard diet fell between 0.05% and 0.3% of the finished food product. The sponsors submit that the consumption of Xanthan gum purified by recovery with ethanol will not reach the levels suggested above, because the assumptions used to generate the estimates were very conservative. Manufacturers of Xanthan gum purified by recovery with ethanol are limited, and currently, there are none in the United States. Further, Xanthan gum purified by recovery with ethanol is not expected to have technical advantages that would result in its use in all foods within a given category. Nonetheless, all feeding studies conducted on humans and various species of animals have demonstrated that Xanthan gum is safe, even when consumed at relatively high levels [Eastwood, 1993 Vol 2 Tab 22 and Woodard, *et al.*, 1973 Vol 4 Tab 81].

Xanthan gum purified by recovery with ethanol has been shown to have lower alcohol residual levels than when purified by recovery with isopropanol. This may gradually open up the market for using Xanthan gum for its functions as a dietary fiber and/or dietary supplement. Estimates have been given that per capita daily intake for these purposes could reach 1.5 to 4.5 grams per day at some point in the future.

The commercial Xanthan gum product currently being produced or sold in the U.S.

Xanthan gum purified by recovery with ethanol does not present a novel process to the food manufacturing industry. Ethanol as an extractant, and as an ingredient in foods has been commonly used for many decades in food processing. The flavor enhancing effects of ethanol are used in enriched-miso fermentations; canned Atlantic sea scallop meats contain ethanol; and, common household flavoring products, such as vanilla often contain ethanol as a diluent in relatively high concentrations (35% on a volume basis) [Chiou *et al.*,1999 Vol 2 Tab 14, McLachlan, *et al.*, 1999 Vol 3 Tab 42, and McCormick Product Label, 2001 Vol 3 Tab 41].

Ethanol purification is also known in the food processing industry. For example, ethanol is also approved for the recovery of Xanthan gum in the EU. In 1999 at the 53rd meeting, JECFA approved new specifications for Xanthan gum, which include levels of ethanol at not more than 500 mg/kg [JECFA, 1999 Vol 3 Tab 34].

Residual ethanol analyses on Xanthan gum purified by recovery with ethanol have shown that the maximum ethanol content determined was 12 ppm [ISI Raw Data, 2001 Vol 3 Tab 32]. The sponsors of this GRAS Report submit that this level is well below that permitted for Xanthan gum in the EU, and is also well below other incidental sources of ethanol in the diet.

C. Safety Evaluation of Xanthan Gum in Animals

Woodard, *et al.*, 1973 published a review of the safety studies conducted earlier on Xanthan gum [Woodard, *et al.*, 1973 Vol 4 Tab 81]. These included a two-year feeding study in rats and dogs and a three-generation reproduction study in rats.

Xanthan gum Lot No. SS-2667, produced from *Xanthomonas campestris* var. wm-3.2 was used in the studies described below. The analysis of the test substance showed that it contained: moisture, 15.5 %; ash, 8.76 %; nitrogen, 0.98 %; arsenic (As_2O_3), 0.1 ppm; lead, 0.1 ppm; total bacteria, 500 colonies/g; zero coliforms, and no viable *Xanthomonas campestris* [Woodard, *et al.*, 1973 Vol 4 Tab 81].

1. Two-Year Rat Feeding Study

In the first study four groups of 30 male and 30 female weanling Charles River CD strain rats received Xanthan gum at levels in the basal diet (Purina Laboratory) 0, 0.25, 0.50 and 1.0 g/kg b.w./day for 2 years. Animals were housed individually in temperature-controlled quarters with food and water available at all times. Concentrations in the diet were adjusted 4 times in order to maintain a constant level of compound intake in terms of g/kg b.w./day. The rats were weighed weekly, and food intake was measured at that time. Complete hemograms including thrombocyte count and coagulation time, and biochemical determinations consisting of blood glucose, prothrombin time and serum glutamic-pyruvic transaminase were determined for 5 male and 5 female rats from the control group and the 1.0-g/kg b.w.group throughout the study, and for 5 male and 5 female rats from each treatment group at the termination of the study. Gross necropsies were performed for all rats that survived the experiment as well as for any found dead or sacrificed in moribund condition. Tissues were examined microscopically from approximately 50 rats from each treatment group from liver, kidney, heart, spleen, lung, adrenal, thyroid, pituitary, lymph node, uterus or prostate, stomach, small intestine, pancreas, urinary bladder, brain, marrow, skeletal muscle, eye, tissue mass, colon and mammary glands.

Survival was reported to be comparable for both the treatment and control groups. The investigators reported that most deaths occurred after 78 weeks, and that survival at 104 weeks was 48, 52, 63, and 52% respectively for the control 0.25, 0.50 and 1.0 g/kg b.w./day groups. Other results showed that no significant effect on growth rate, hematologic values, organ weights or tumor incidence occurred as the result of Xanthan gum consumption. Soft stools were noted more frequently for the high- and middle-level males, but the differences from the control group barely reached the level of statistical significance according to the authors [Woodard, *et al.*, 1973 Vol 4 Tab 81]. The differences among the groups with respect to the numbers of neoplasms observed were not significant (See Table 32).

Table 32
Number of Neoplasms Observed in Rats After a Two-Year Feeding of Xanthan Gum

	Dosage level (g/kg b.w./day)			
Neoplasms	Control	0.25	0.50	1.0
Number of rats per group	36	47	43	39
Pituitary	19	27	24	21
Uterine	2	0	0	5[a]
Mammary	5	12	10	8
Other	13	7	14	13
Total	39	46	48	47

[a] All polyps.

2. Two-Year Dog Feeding Study

Groups of four male and four female beagle dogs, 4-8 months of age, from one supplier, were fed Xanthan gum in the daily basal diet at the levels of 0.0, 0.25, 0.37 and 1.0 g/kg b.w./day, Seven days per week for 107 weeks. Diets for each treated group were made twice weekly. Each ration was supplemented with 45 g of canned beef during the first 10 weeks of the study. Thereafter, 120 ml of canned evaporated milk was added instead of the meat [Woodard, *et al.*, 1973 Vol 4 Tab 81]. For each 200 grams of the daily basal ration 440 ml of water was added, except for the diet containing 1.0 g/kg b.w./day. This diet required 600-800 ml of water.

Dogs were evaluated for survival, body weights, eye examinations, hemograms, including platelet counts and thrombocyte counts, electrocardiograms, heart rates, blood pressures, and neurologic examinations. Clinical chemistry determinations consisted of blood urea nitrogen, serum alkaline phosphatase, serum glutamic oxaloacetic and -pyruvic transaminases, prothrombin time and serum calcium. Careful examinations of the stool were made in regard to color, weight, consistency and pH. Urinary excretions were measured, and qualitative urinalyses were determined. All dogs were sacrificed after 107 weeks and were subjected to gross necropsy. Liver, kidney, heart, spleen, lung, adrenal, thyroid, pituitary, gonads, uterus or prostate and brain were weighed. These organs plus trachea, esophagus, lymph node, salivary gland, thymus, stomach, small intestine, pancreas, colon, cecum, urinary bladder, spinal cord, bone marrow, skeletal muscle, mammary glands, skin, eye, gall bladder, and nerve were subjected to histopathologic observations.

At the end of the study, treated dogs were comparable to control dogs in weight, hemograms, blood pressure, behavior, gross and microscopic tissue examinations and gross and relative organ weights [Woodard, *et al.*, 1973 Vol 4 Tab 81]. Serum alkaline phosphatase, prothrombin time, blood glucose, and serum glutamic-oxaloacetic and -pyruvic transamiases showed no changes. Blood urea nitrogen was elevated at several test intervals for one male in the 1.0-g/kg b.w.group.

Stools of control dogs were most generally described as formed. However, during the first year of the study one-third of the observations described control stools as a spread-to-formed consistency; the remainder were described as formed. During the first year on the study, the stool consistency for dogs receiving 1.0 and 0.37 g/kg b.w.of Xanthan gum was frequently described as "cake batter consistency." The color was light brown with a glossy appearance. The consistency became more dense (pudding like) the second year of the study. For dogs receiving 0.25 g/kg, stools were divided approximately half formed and half of a spread-to-formed consistency. The color of the stools at this level was darker than those of the 1.0- and 0.37-g/kg b.w.dogs and was not glossy [Woodard, *et al.*, 1973 Vol 4 Tab 81].

The weight of feces excreted showed a dose-related increase for treated dogs. At week 50 the average weight of feces excreted each day was 138, 200, 273 and 289 g, respectively for the control, 0.25, 0.37 and 1.0 g/kg b.w./day groups. The authors stated that the increased weight was most likely a reflection of the known capacity of Xanthan gum to absorb water [Woodard, *et al.*, 1973 Vol 4 Tab 81].

Qualitative urinalyses performed on cage-collected urine samples showed a more frequent presence of 1+ urinary albumin for 1.0-g/kg b.w./day dogs than for control dogs. The urinary specific gravity was increased over control values for dogs in each treatment group and appeared to be dosage related. These values ranged 1.014-1.022, 1.021-1.040, 1.023-1.037 and 1.027-1.041 for control, 0.25, 0.37 and 1.0 g/kg b.w./day levels, respectively.

Ophthalmoscopic examinations revealed a bilateral grayish, and sometimes gray spotting or mottling of the *tapetum lucidum* for 9 dogs. The mottling was observed for one dog in each of the control, 0.37- and 0.25-g/kg b.w.groups and for six dogs in the 1.0 g/kg b.w./day group. Four of these dogs, one in the control, two in the 1.0-g/kg b.w. group, and one in the 0.37-g/kg b.w. group were littermates. The histopathologic examination of the eye sections did not show abnormality. Therefore, these findings were believed to be some normal anatomical variation [Woodard, *et al.*, 1973 Vol 4 Tab 81].

Blood urea nitrogen was elevated at several test intervals for one high-level male. A dose-related increase in fecal weight, an increase in the specific gravity of the urine and a more frequent presence of urinary albumin for dogs in the 1.0-g/kg b.w./day group was noted [Woodard, *et al.*, 1973 Vol 4 Tab 81].

3. Rat Reproduction Study

Xanthan gum was fed in the diet to groups of 10 male and 20 female albino rats of the first generation and to groups of 20 male and 20 female albino rats of the two successive generations at levels providing daily dosages of 0.0, 0.25 and 0.5 g/kg b.w.of body weight per day. Pairs of rats were mated to produce two litters per generation, with the next generation selected from weanlings of the second litters [Woodard, *et al.*, 1973 Vol 4 Tab 81].

Criteria evaluated for the groups of rats were survival mean body weights, general appearance, behavior, and reproductive performance. In addition, females that had fewer than two litters were examined for uterine implantation sites to determine any fetal resorptions.

Criteria evaluated for the litters were number of litters per group, numbers of births and stillbirths, physical condition, mean weights at birth and weaning, and percent young alive at weaning. Also evaluated were malformations of offspring; autopsy observations on litters of the second and third generations; body, liver, kidney, and heart weights on weanlings of the second, F_3b litters of the third generation, histopathologic observations on tissues of weanlings in each control and the high dose (0.50 g/kg) F_3b litter [Woodard, *et al.*, 1973 Vol 4 Tab 81].

The parent rats receiving Xanthan gum and the control groups of rats were comparable in survival, general appearance, and reproductive performance. Body weights of treated groups were slightly less than those of control parents during each generation. Corresponding test and control litters of the three generations were comparable in number of litters per group, numbers of live births, physical condition, mean weight at birth and weaning, percent young alive at weaning, and gross autopsy observations.

Examination of females of the F_0 and F_1b generations that had fewer than two litters for uterine implantation sites revealed no effect on fetal resorption related to Xanthan gum administration. No malformations were observed in any of the offspring. Gross autopsy observations for the test and control, F_2, and F_3 litters were comparable. Body, liver, kidney, and heart weights of test and control weanlings of the F_3b litters were comparable. No effect of Xanthan gum administration was detected by histopathological observations on tissues of weanlings in each control and high dietary level (0.50 g/kg b.w./day) F_3b litter [Woodard, *et al.*, 1973 Vol 4 Tab 81].

The authors concluded that dietary feeding of Xanthan gum at levels of 0.0 (control), 0.25, or 0.50 g/kg b.w./day to groups of albino rats of both sexes during a three-generation reproduction study had no effect on reproduction as judged by all the criteria evaluated for the groups of rats and for the litters. Table 33 summarizes the reproductive results of the three-generation study.

Table 33

Three-Generation Reproductive Study on Rats Fed Xanthan Gum In The Diet

No. of females mated	Diet Level (g/kg)	No. of litters	Live births	Still-births	Litter size	Birth weight (g)	% Live weaning	Weanling weight (g)
	F_1a Generation							
20	0	16	134	3	8	5.7	91.8	29.0
20	0.25	14	132	9	9	6.0	60.6	29.2
20	0.50	17	157	0	9	5.9	82.2	32.1
	F_1b Generation							
19	0	16	132	1	8	6.9	81.8	33.7
18	0.25	15	133	0	9	7.1	90.2	34.7
19	0.50	18	172	2	9	6.6	88.4	31.3
	F_2a Generation							
20	0	17	165	13	10	6.1	69.7	31.7
21	0.25	17	181	5	11	6.1	62.4	29.9
22	0.50	21	210	4	10	6.1	86.7	33.0
	F_2b Generation							
19	0	13	140	4	Al	6.1	71.4	34.0
20	0.25	19	214	7	11	6.3	78.0	31.4
22	0.50	21	237	7	-11	5.9	74.7	34.2
	F_3a Generation							
22	0	16	129	2	8	6.1	78.0	36.7
22	0.25	18	158	2	9	6.2	66.0	30.7
22	0.50	16	139	14	9	5.9	70.0	33.1
	F_3b Generation							
22	0	18	157	12	9	6.6	85.0	41.2
22	0.25	16	156	1	10	6.7	67.9	36.3
22	0.50	18	159	6	9	6.4	76.1	41.5

[a]Mean values.

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4. Specific Comments Noted in the Review

The authors of the review provided specific comments on the findings of the three animal toxicology studies. They indicated that the results for the two-year rat and three-generation reproduction studies showed no negative effects with respect to the feeding of Xanthan gum. The increased incidence of soft stools, although not statistically significant, could be attributed to the physicochemical properties of Xanthan gum. This effect is now generally recognized to occur in animal studies when undigested or partially digested polysaccharides are fed at levels in excess of 2.5 to 5.0% of the diet. The investigators indicated that this effect was more clearly seen in the dog and was reflected in a dosage-related increase in fecal weight and a compensatory increase in specific gravity of the urine. The survival rate, occurrence of spontaneous tumors and reproductive performance in rats compared favorably with data reported in the literature for comparable numbers of animals studied for similar periods of time but using rats from other sources [Woodard, *et al.*, 1973 Vol 4 Tab 81].

D. The Dietary Effects of Xanthan Gum In Man

Studies on the effect of Xanthan gum in man have been mainly centered around determining the mechanism by which it acts as a bulking agent, or as an aid in the control of blood glucose or cholesterol levels. Significant from a safety and tolerance point of view, is that in the human studies which have been published, the protocol called for the consumption of ten to fifteen grams of Xanthan gum per day (several times the exposure estimated in Section II of this Report for those consuming a normal diet). None of the studies reported a lack of tolerance or severe gastrointestinal stress. One longer term study, in which twelve grams per day of Xanthan gum was fed as an ingredient in muffins (two grams per muffin) reported that the subjects felt fullness, but did not experience any other distress [Osilesi *et al.*, 1985 Vol 3 Tab 48]. The physiological effects of Xanthan gum in humans as reported in these studies will be briefly reviewed in this section.

Eastwood, 1987 researched the dietary effects of Xanthan gum in humans. Following a 7-day control period, 5 male volunteers consumed Xanthan gum, on each of 23 consecutive days. The volunteers consumed between 10.4 g and 12.9 g of Xanthan gum daily, depending on body weight. Measurements before and at the end of the test period showed that the ingestion of Xanthan gum, as a pre-hydrated gel, acted as a bulking agent in terms of its effects on fecal wet and dry weight and intestinal transit time, but had no significant effect on plasma biochemistry, hematological indices, urinalysis parameters, glucose tolerance and insulin tests, serum immunoglobulins, triglycerides, phospholipids and HDL cholesterol, breath hydrogen and breath methane concentrations [Eastwood, 1987 Vol 2 Tab 23].

The authors reported a moderate (10%) reduction in serum cholesterol and a significant increase in fecal bile acid concentrations. The data indicated that the ingestion of Xanthan gum caused no adverse dietary nor physiological effects in any of the subjects. In particular, all of the enzymatic and other parameters that act as sensitive indicators of adverse toxicological effects remained unchanged [Eastwood, 1987 Vol 2 Tab 23].

Following the work of Eastwood in the late 1980s, several studies tried to correlate the *in vitro* determinants of the bacterial breakdown of Xanthan gum with the effects of actually feeding the gum on colonic function in man [Tomlin, *et al.,* 1988 Vol 4 Tab 69, Edwards, *et al.,* 1987 Vol 2 Tab 24, and Daly, 1993 Vol 2 Tab 18]. The purpose of these studies was to try to develop a method that would predict the function (or best application) of a given dietary fiber, when consumed by humans.

For example Daly, *et al.,* stated that complex non-starch polysaccharides are widely used as bulking and laxative agents, but various polysaccharides were found to differ in their potency. Several researchers in the field had postulated that these differences might be related to the ability of the polysaccharide matrix to retain water [Daly, 1993 Vol 2 Tab 18]. However, certain studies had suggested an inverse relationship between water-holding capacity and stool output [Stephen et al., 1979 Vol 4 Tab 63]. Therefore, correlations between the *in vivo* and *in vitro* findings in many fiber studies did not substantiate the hypothesis that the *in vivo* effect of a given polysaccharide could be predicted from its fermentation characteristics *in vitro* [Daly, 1993 Vol 2 Tab 18]. The explanation for this apparent paradox ultimately was shown to lie in the fact that it is the water-holding capacity after colonic fermentation that is more directly related to the effect of a polysaccharide on stool output, and many well-known polysaccharides with the highest water-holding capacities are rapidly fermented, leaving them with little water-holding capability in the colon. For instance, polysaccharides such as guar gum that are readily broken down by colonic bacteria have only a small effect on stool output, while isphagula which resists bacterial degradation is a much better laxative [Prynne, *et al.*, 1979 Vol 4 Tab 52 and Cummings, *et al.* 1978 Vol 2 Tab 16].

According to Osilesi *et al.*, 1985, Xanthan gum has a coiled backbone and tightly packed side chains. The tight packing would tend to resist bacterial penetration and could account for its relatively poor fermentation by colonic bacteria. Thus, the maintenance of the polysaccharide structure with retention of the water incorporated in the matrix would theoretically lead to a better laxative effect [Osilesi *et al.*, 1985 Vol 3 Tab 48].

Tomlin and Read, 1988, investigated the effects of three different soluble polysaccharides (guar gum, isphagula, and Xanthan gum) on fecal mass and whole-gut transit time on seven healthy volunteers, who consumed 14-15 grams per day for seven days [Tomlin, *et al.,* 1988 Vol 4 Tab 69]. These results were compared with the results of *in vitro* incubations of the polysaccharides with fecal samples collected from each of the volunteers before consumption of the polysaccharides. In this study it was found that a greater increase in fecal mass was produced when some of the polysaccharide structure was preserved following fermentation. Structural retention was determined by incomplete removal of viscosity. In other words, retention of some viscosity was associated with an increase in stool weight, while reduction in viscosity and evidence of fermentation was associated with reductions in transit time. In certain ways this was an important study, which showed that transit time and stool output could be effected independently by the feeding of viscous polysaccharides [Tomlin, *et al.,* 1988 Vol 4 Tab 69]. With respect to Xanthan gum specifically, the authors found considerable individual variation in the ability of the unadapted fecal cultures to ferment it. In conclusion the authors suggested that the major mechanism by which soluble polysaccharides increase fecal mass is through water retention, as opposed to stimulation of bacterial growth.

A 1993 study by Daly, *et al.,* reviews the findings of several previous studies, and its results are illustrative of the typical physiological effects reported for Xanthan gum [Daly, 1993 Vol 2 Tab 18]. In that study 15 g/d of Xanthan gum was given for 10 days to eighteen normal volunteers. These were eighteen healthy male volunteers who were aged between 19 and 34 years and had a normal weight-for-height ratio. None of the subjects had taken any antibiotics during the previous 6 months. All volunteers gave written informed consent for the study to be carried out, and the protocol was approved by the local Ethical Committee (Sheffield Area, Trent Region Health Authority).

Xanthan gum (Keltrol T; Kelco International Ltd, San Diego, CA, USA) was supplied to each subject in 5 g individual portions to be taken three times daily with meals. In order to maximize the palatability of the Xanthan gum and to simulate its mode of ingestion in foodstuffs the preweighed 5 g portions were stirred into 150 ml boiling water in which three cubes of jelly (Rowntrees Ltd, York) had previously been dissolved. This mixture was made up in 150 ml plastic pots fitted with lids and then placed under refrigeration for 24 h to give a palatable, fully hydrated, flavored substance with a thick jelly-like consistency.

In vivo measurements of stool output, transit time, frequency of defecation and flatulence were compared with a preceding control period of 10 days. At the end of the control and test periods fresh fecal homogenate from each subject was anaerobically incubated with Xanthan gum and control solutions to assess the ability of the bacteria to break down the gum. Xanthan gum was found to be a highly efficient laxative agent causing significant increases in stool output ($P < 0.01$), frequency of defecation ($P < 0.05$) and flatulence ($P < 0.01$), while having variable effects on transit time [Daly, 1993 Vol 2 Tab 18].

Before feeding, fecal samples from twelve of the eighteen subjects could reduce the viscosity of the gum *in vitro,* indicating that the native colonic microflora of those subjects were capable of partially degrading the gum. After the subjects consumed Xanthan gum for ten days, sixteen of the eighteen fecal samples could reduce Xanthan gum viscosity. These samples also produced significantly greater amounts ($P < 0.05$) of hydrogen and short-chain fatty acids, indicating bacterial adaptation in the presence of the substrate [Daly, 1993 Vol 2 Tab 18].

During the initial days of increasing fiber consumption, humans may experience a variety of gastrointestinal symptoms, including greater than normal gas production. In an Expert Opinion Dr. George C. Fahey of the University of Illinois stated that if humans consumed a high quantity of polysaccharide, gas production during the early stages of consumption might occur. "But, colonic microbes usually will adapt to high level fiber feeding after 4-7 days, after which gas production will be reduced to normal levels. In addition, humans may adapt to high level fiber feeding by gradually increasing their fiber consumption over a 7 day period. This generally will circumvent the problem outlined above." Xanthan gum should be no different than any other fiber in this regard [Fahey, Expert Opinion, 2001 Vol 2 Tab 27].

This appears to be the case with Xanthan gum. As will be discussed in more detail later in this Section, Osilesi *et al.*, 1985 fed 12 grams of Xanthan gum per day for six weeks to nine diabetic and four non-diabetic subjects. The Xanthan gum was incorporated into muffins (six muffins per day; 2.0 grams per muffin), and the authors reported that the subjects had no difficulty in consuming the muffins, even though, they were described as somewhat gummy and chewy. More importantly, however, while the subjects did report a feeling of "unusual fullness" none complained of diarrhea or severe gastrointestinal distress [Osilesi, *et al*, 1985 Vol 3 Tab 48]. It may be worth noting that in the Osilesi, *et al*, study, the subjects not only consumed Xanthan gum in smaller increments than in the Daly study (two grams versus five grams), but they also consumed the gum in the presence of a more complex food matrix, i.e., muffins versus a Jello™-like substance.

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In the Daly, et al., study, ingestion of five grams of Xanthan gum three times per day for 10 days caused a significant increase in stool weight that was composed of significant increases in both stool dry and wet weights. Interestingly, the percentage of stool water remained unchanged. This result was observed for all but three subjects. Stool frequency was also increased as was the average weight of each stool [Daly, 1993 Vol 2 Tab 18].

Ingestion of Xanthan gum did not cause any significant change in transit time. Nine subjects experienced increased transit time while nine had decreased values. The net effect of this was a reduction in the spread of results so that subjects with the slowest transit times showed an acceleration with Xanthan gum, while those with short transit times showed a delay. Table 34 below presents these results.

Table 34

The Effect of Ingestion of Xanthan Gum by Healthy Male Subjects* (15 g/d)

In Vivo Variables	Control	SE**	Xanthan	SE**	Statistics
Transit time (h)	46.7	4.9	40.7	2.8	NS
Stool wt (g/d)	190.3	13.6	242.8	15.8	P < 0.001
Stool water (g/d)	127.4	9.9	175.9	13.4	P < 0.035
Stool dry wt (g/d)	53.2	4.1	63.4	3.3	P < 0.008
Stool wt (g/stool)	169.5	12.4	195.8	18.0	P < 0.049
Water in stool (%)	71.3	1.5	72.6	1.4	NS
Stool frequency (/d)	1.14	0.08	1.31	0.08	P < 0.035
Flatulent episodes (/d)	9.6	2.2	13.9	2.5	P < 0.001

*NS = not significant

**SE= Standard error of the mean

Xanthan gum caused a highly significant increase in flatulence in all but one subject ($P < 0.001$). Incubation of Xanthan gum with fecal bacteria from each of the subjects at the end of the control period caused a reduction in viscosity in samples from twelve of the eighteen subjects but viscosity was eliminated in only seven of these (See Table 37). Incubation with Xanthan gum also reduced the pH of the culture, increased the production of short chain fatty acids (SCFAs) and, produced H_2, but these changes were not significantly different from those seen when the feces were incubated with the same volume of medium [Daly, 1993 Vol 2 Tab 18].

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The author reported that after the subjects had consumed Xanthan gum for 10 days, their fecal microflora had adapted, so that they had greater ability to ferment Xanthan gum. This phenomena was evidenced by a significant increase in hydrogen gas production, when a medium containing Xanthan gum was inoculated with the subject's fecal material (see Table 35). Given that the viscosity of Xanthan gum is dependent on its highly branched –structure, a reduction in viscosity would be associated with degradation of the molecule. Samples from all except two of the subjects reduced the viscosity of the Xanthan gum and eleven samples completely eliminated the viscosity. The average reduction in viscosity was greater than before Xanthan gum was fed (50.8 mPas v. 24.1 mPas; P = 0.045) and the amounts of H_2 and SCFA produced had become significantly greater than those with the feces alone (P = 0.049) [Daly, 1993 Vol 2 Tab 18].

Table 35
The Effect of Feeding Xanthan Gum to Healthy Male Subjects on Properties of Feces Incubated *In Vitro*

Incubation	Xanthan gum Medium[1, 5]		Xanthan gum Feces[2, 5]		Feces Medium[3, 5]	
	Mean	SE[4]	Mean	SE[4]	Mean	SE[4]
Before feeding						
Final viscosity (mPas)	27.4	17.1	16.4	16.1*	1.6	0.06
Final pH	6.83	0.01	6.74	0.02	6.77	0.02
H_2 production (μl)	5.4	0.03	31.2	9.0*	52.0	26.6
SCFA production (mmol/kg stool)	0	0	2632	336*	2316	390
After feeding						
Final viscosity (mPas)	35.8	17.0	17.1	23.3*	1.84	0.08
Final pH	6.86	0.03	6.77	0.02	6.79	0.03
H_2 production (μl)	1.83	1.2	102.5	30.5*	63.5	38.6
SCFA production (mmol/kg stool)	0	0	2656	388*	2111	242

*P <0.05: Mean values were significantly different from those for Xanthan gum-medium.
*P <0.05: Mean values were significantly different from those for feces-medium.
[1] Xanthan gum-medium contained 15 ml of Xanthan gum solution (2.5g/l) + 15 ml of medium.
[2] Xanthan gum-feces contained 15 ml of Xanthan gum solution (2.5g/l) + 15 ml of fecal homogenate.
[3] Feces medium contained 15 ml of fecal homogenate + 15 ml of medium.
[4] Standard Error of the Mean
[5] The medium consisted of NaCl (60mM), $NaHCO_3$ (40mM), KCl (10mM), and tryptone (10g/l), all dissolved in distilled water.

IV. Detailed Summary , (continued)

The investigators noted that before the subjects were fed Xanthan gum, there was an inverse relationship between transit time and stool weight (P = 0.001). After ingesting the Xanthan gum the two variables changed independently of each other (P = 0.192), but the inverse relationship was still present after the feeding period (P = 0.001) [Daly, 1993 Vol 2 Tab 18].

There were no statistically significant associations between the reduction in viscosity during fermentation and either the changes in stool weight or the reductions in transit time. A similar percentage of those subjects that increased stool weight and those that failed to increase stool weight after feeding Xanthan gum retained some viscosity after fermentation (53% v. 66%). Also a similar percentage of the subjects who increased or who reduced transit times showed some reduction in viscosity in *vitro* (56% v. 44%). Both the production of H_2 and the reduction in viscosity *in vitro* were significantly associated with an increase in stool frequency (P = 0.04). There were also significant associations between a fall in pH and both decreases in transit time (P = 0.041) and increases in flatulence (P = 0.035) [Daly, 1993 Vol 2 Tab 18].

The findings from this study showed that Xanthan gum is a highly efficient stoolbulking agent causing an average increase in fecal output of about 50 g/d when given at a dose of 15 g/d, confirming the previous findings of Eastwood *et al.,* 1987. The increase in mass results not only from an increased amount of water, but also from a significant *(P = 0.008)* increase in dry weight. Ingestion of Xanthan gum was also shown to cause a significant increases in the frequency of defecation (P = 0.035) and weight of each bowel movement (P = 0.049).

The effect on transit time in this study, unlike other polysaccharides was not significant, although on average it decreased. In fact the Xanthan gum appeared to have a normalizing effect similar to that shown by Payler *et al.,* 1975, with wheat bran [Tomlin, *et al.,* 1988 Vol 4 Tab 69 and Daly, 1993 Vol 2 Tab 18].

The results of the study only partly supported the author's previous observations that accelerations in transit were associated with a reduction or abolition of viscous properties, suggesting a partial or complete bacterial degradation. A reduction in viscosity was not associated with an acceleration of transit time, though it was associated with an increased stool frequency. Additionally, acceleration of transit time was not associated with production of SCFA and H_2, but it was associated with a drop in pH. H_2 production was correlated with increased stool frequency [Daly, 1993 Vol 2 Tab 18].

The investigators concluded that after feeding Xanthan gum for 10 days, significantly greater reductions in viscosity and significantly greater amounts of both H_2 and SCFA were produced by the fecal cultures compared with the control; this increase in the physical breakdown of the gum linked to increased amounts of fermentation products indicated a greater degree of fermentation. The increase in breakdown of the polysaccharide structure after a period of exposure to bacteria could simply be due to increasing numbers of bacteria or, more likely, the bacteria may have altered their metabolism in order to derive energy more efficiently from the gum. This increase in enzymic activity has been shown previously with oat bran (Chang *et al.*1979) and wheat bran (Bourke & Neale, 1980) on exposing bacteria to the substrate. The ability of the bacteria to adapt may offer one explanation for the loss of efficiency of laxatives reported by many consumers [Daly, 1993 Vol 2 Tab 18].

Although this study confirmed that Xanthan gum is a highly effective bulk laxative, it also demonstrated that it is not possible to predict the physiological effect of a polysaccharide solely from its fermentation characteristics *in vitro* (Adiotomre *et al.* 1990) [Daly, 1993 Vol 2 Tab 18]. However, a reduction in colonic pH, and an increase in the production of SCFA have been associated with increased health of the digestive tract., supporting the assumption put forth in Section II, Table 10 that Xanthan Gum purified by recovery with ethanol has potential as a dietary supplement.

E. *In Vitro* Fermentation Studies in Animal Models

Sunvold *et al.,* and his colleagues at the University of Illinois used dogs as a model to study *in vitro* fermentation of selected fiber sources and *in vivo* digestion and metabolism of fiber-supplemented diets. Two experiments were conducted to evaluate single sources and blends of dietary fiber in dog food. In a first experiment, 14 fibrous substrates were fermented *in vitro* using dog feces as the source of innoculum. Results showed that organic matter disappearance was lowest ($P < .05$; < 10%) for alpha cellulose (Solka Floc) and oat fiber and greatest ($P < .05$; > 80%) for fructooligosaccharides and lactulose. Solka Floc, oat fiber, gum karaya, and Xanthan gum produced the least ($P < .05$; < 1 mmol/g of substrate organic matter) total short-chain fatty acids (SCFA). Lactulose, citrus pectin, and guar gum produced the greatest ($P < .05$; > 6.8 mmol/g of substrate organic matter) total SCFA [Sunvold *et al.,* 1995 Vol 4 Tab 65].

In this study Xanthan gum showed an organic matter disappearance of 28% after 24 hours. It was fermented mainly to acetate, with only minor quantities of propionate and butyrate being produced in this dog model.

F. Xanthan Gum Fermentation as a Dietary Fiber in the Management of Diabetes Mellitus

Vinik, *et al.,* has reported that persons with diabetes could benefit by the addition of at least 25 grams of soluble fiber /1000 kcal [Vinik, *et al.,* 1988 Vol 4 Tab 78]. Xanthan gum was fed in muffins (12 g/day) during either the first or second half of a 12-week feeding period to free-living human subjects. Nine subjects were diabetic, having moderately elevated serum glucose but managing without insulin or hypoglycemic drugs, and four were nondiabetic controls. The test subjects consumed the Xanthan gum in six muffins each containing two grams of the gum, and the muffins were eaten as a substitute for the bread group; i.e., two with the main meal, and the remainder with other meals and with other foods [Osilesi, *et al*, 1985 Vol 3 Tab 48]. Control subjects consumed the same type of muffin, but without the Xanthan gum.

Before the study and near the end of weeks 3, 6, 9, and 12, fasting blood samples were taken in the morning and again two hours after an oral glucose load of 1 g/kg b.w. The blood samples were analyzed for glucose, insulin, gastrin, and gastric inhibitory polypeptide (GIP). Triglycerides and total cholesterol were determined in total plasma from the fasting blood, as were high-density lipoprotein (HDL), low-density lipoprotein (LDL) and very-low-density lipoprotein (VLDL) [Osilesi, *et al*, 1985 Vol 3 Tab 48].

The study showed that feeding of Xanthan gum for three or six weeks induced a marked reduction in fasting serum glucose in diabetic subjects. At the end of the six-week period fasting glucose was nearly 40% lower in comparison to the period when control Xanthan-free muffins were consumed. The consumption of Xanthan gum reduced circulating total plasma cholesterol in the LDL (by 13%) and VLDL fractions (by 41%) in diabetic subjects. Additionally, total triglycerides were reduced by 25% (125 ± 31 versus 167 ± 65 mg/dl), and VLDL triglycerides were reduced by 28% (71 ± versus 99 ± 41 mg/dl). Xanthan gum also tended to lower fasting and postload levels of gastrin and GIP. Subjects reported a sense of fullness after consuming Xanthan muffins but no severe digestive symptoms were noted [Osilesi, *et al*, 1985 Vol 3 Tab 48].

G. Food Safety of Ethanol and Xylitol Manufactured as Co-products

Section III of this report discussed examples of the processing of various foods with ethanol. This section presents an example of the co-production of ethanol with xylitol. Xylitol is an approved food additive, whose regulation appears at 21 CFR § 172.395. As a food additive, FDA would have reviewed the manufacture of this product prior to its approval. Since the agency placed no limits on the manufacture of xylitol, presumably the contact with and subsequent purification of xylitol from ethanol would not be deemed a health hazard. In a study published by Latif *et al.*, 2001, *Saccharomyces cerevisiae* and *Candida tropicalis* were used separately and as co-cultures for simultaneous saccharification and fermentation (SSF) of 5-20% (w/v) dry corn cobs. A maximal ethanol concentration of 27, 23, 21 g/l (w/v) from 200 g/l (w/v) dry corn cobs was obtained by *S. cerevisiae, C. tropicalis* and the co-culture, respectively, following 96 hours of fermentation. However, the authors reported that theoretical yields of 82%, 71% and 63% were observed from 50 g/l dry corncobs for the above cultures [Latif *et al.*, 2001 Vol 3 Tab 38].

Maximal xylitol concentrations of 21, 20 and 15 g/l from 200 g/l (w/v) dry corn cobs were obtained by *C. tropicalis*, co-culture, and *S. cerevisiae*, respectively, compared to maximum theoretical yields of 79.0%, 77.0% and 58% from 50 g/l of corn cobs. The investigators found that volumetric production for ethanol and xylitol increased with an increase in substrate concentration, whereas, yield decreased. Use of *S. cerevisiae* and *C. tropicalis* as individual cultures resulted in better product yields (0.42 and 0.36 g/g) for ethanol and (0.52 and 0.71 g/g) for xylitol, respectively, whereas, the co-culture showed moderate levels of ethanol production (0.32 g/g) in conjunction with almost maximal levels of xylitol (0. 69 g/g). These results indicated that ethanol was present at levels approximating 64% of those of xylitol, which was subsequently successfully purified from the alcohol [Latif *et al.,* 2001 Vol 3 Tab 38].

H. Safety of Ethanol as a Food Grade Purification Agent

The safety of ethanol, CAS #64-17-5 has been studied extensively, both when taken orally, and when applied to the skin. The following information indicates that toxic levels of ethanol far exceed any residual ethanol that may be encountered in the consumption of Xanthan gum purified by recovery with ethanol. In Section II of this Report, the sponsors reported that consumption of Xanthan gum purified by recovery with ethanol would not expose the consumer to more than microgram quantities of ethanol, even when Xanthan gum was consumed at the estimated 90th percentile (Table 36).

Employees at the ZZBP manufacturing facility may also be exposed to ethanol through inhalation, the eyes, and the skin. The following information addresses acute and chronic levels for those routes of exposure. However, there is no evidence from either plant monitoring of ethanol vapor levels or from worker health records to indicate these acute or chronic levels of exposure have ever been encountered.

Acute ethanol inhalation exposure for humans is considered to be in the range of 1000-10,000 ppm. This level has caused temporary irritation of the upper respiratory tract and coughing; and if exposure is continued, central nervous system depression with headache, stupor, fatigue, dizziness, drowsiness, dullness, lassitude and loss of appetite. A level of 20,000 ppm has been considered just tolerable, and above this level the atmosphere has been described as intolerable, causing suffocating on even brief exposures. Additionally, exposure of animals to various concentrations has resulted in excitation followed by ataxia, incoordination, prostration, twitching, general paralysis, dyspnea, and occasionally death due to respiratory failure [Egland, *et al.*, 2001 Vol 2 Tab 25].

Chronic inhalation exposure to ethanol is described as repeated or prolonged inhalation of vapors that may cause irritation of the mucous membranes, headache, dizziness, nervousness, tremors, fatigue, nausea, narcosis, lack of concentration, and somnolence. Tolerance may be a factor in individual response to a given air concentration. Reproductive effects have been reported in animals [Egland, *et al.*, 2001 Vol 2 Tab 25].

Acute eye exposure to vapor concentrations of 1000-10,000 ppm may cause temporary eye irritation, with 15,000 ppm causing continuous lacrimation. Direct contact with the liquid may cause immediate burning and stinging, with reflex closure of the lids, tearing, temporary injury of the corneal epithelium, and hyperemia of the conjunctiva. Healing is usually spontaneous and complete. Depending on the concentration, contact with rabbit eyes may cause a response ranging from mild irritation to severe injury. Irrigation of rabbit eyes with a 10% solution for several minutes caused no serious disturbances according to Egland, 2001.

Chronic eye exposure to ethanol is described as repeated application to rabbit eyes of 40-80% solutions caused corneal cloudiness, conjunctival necrosis and loss of corneal epithelium and endothelium, followed by conjunctival hemorrhaging and edema, infiltration and vascularization of the corneal stroma [Egland, *et al.*, 2001 Vol 2 Tab 25].

Acute skin exposure to ethanol may cause mild redness and burning. Sensitization has occasionally been reported to occur in some individuals resulting in allergic contact dermatitis in the form of eczematous eruptions or, rarely, erythematous flush or contact urticaria at the exposed site. Animal studies indicate that, depending on concentration and duration of exposure, varying degrees of irritation may occur ranging from mild to severe.

Chronic exposure to skin requires repeated or prolonged contact. This may cause defatting of the skin, producing a dry, fissured dermatitis, or other symptoms as in acute exposure. A 31 -day modified draize open test study resulted in no irritation in men, whereas an occlusive test resulted in erythema and induration toward the end of the exposure period [Egland, *et al.*, 2001 Vol 2 Tab 25].

Consumers are familiar with exposure to ethanol by ingestion. Acute ingestion may cause emotional lability and decreased inhibitions, with exhilaration, boastfullness, talkativeness, remorse, and belligerency; followed by gradual visual impairment, muscular incoordination, slowing of reaction time, sensory disturbances, and slurring speech. Other symptoms may include flushing of the face, dilated pupils, rapid pulse, nausea, vomiting, sweating, and diuresis. Ingestion of large amounts may cause confusion, disorientation, loss of motor nerve control, shallow respiration, involuntary defecation and urination, drowsiness, stupor, and possibly coma.

Convulsions due to hypoglycemia and shock with hypotension, tachycardia, cold pale skin, hypotherinia, respiratory depression, and decreased reflexes may occur. Death may occur from respiratory or circulatory failure or later from aspiration pneumonitis or pulmonary edema. Recovery may be accompanied by headache, insomnia, gastritis, infection, restlessness, psychoses with uncontrollable fear, and visual, auditory or sustatory hallucinations, exagerated reflexes, tachycardia, and convulsions. Some individuals sensitized by external contact may suffer from a generalized erythema, stomatitis, urticaria, angioedema, morbilliform eruptions, or eczematous dermatitis at sites previously affected by external contact. Reproductive effects have been reported in animals.

Chronic intoxication may result in weight loss, degenerative changes in the liver, kidneys, and brain, gastroeniteritis with anorexia and diarrhea, cirrhosis of the liver. Polyneuritis with pain, motor and sensory loss in the extremities and optic atrophy may occur. Amnesia, tremors, confusion, impaired judgement and loss or impairment of mental acute myopathy with tenderness, aching, edema, and degeneration of the muscles. The heart may be affected, causing palpitations, extrasystole, tachycardia, or other arrhythmias, which may progress to irreversible myocardial fibrosis and circulatory failure. Ethanol has been clearly demonstrated to cause reproductive effects in humans, and reproductive effects have also been reported in animals [Egland, *et al.,* 2001 Vol 2 Tab 25].

Table 36 presents toxicity data for ethanol in comparison to that estimated to be consumed on a daily basis as a residual on Xanthan gum purified by recovery with ethanol, and containing approximately 12 ppm on a dry weight basis.

Table 36
Ethanol Toxicity Data Compared to Xanthan Gum Residuals

* 700 mg/kg oral-man TDLO
* 2000 mg/kg oral-child LDLO
* 50 mg/kg oral-man TDLO
* 1430 g/kg oral-man TDLO
* 256 gm/kg/12 weeks oral-woman TDLO
* 1400 mg/kg oral-human LDLO
* 19,440 mg/kg subcutaneous-infant LDLO
* 4300 mg/kg intraperitoneal-mammal LD_{50}
* 0.13 μg/kg for a 60 kg adult; Estimated mean daily intake of ethanol as a residual on Xanthan gum, as manufactured by ZZBP
* 0.26 μg/kg for a 60 kg adult; Estimated daily intake of ethanol as a residual on Xanthan gum (as manufactured by ZZBP), when consumed at the 90th percentile

TDLO = lowest toxic dose tested
LDLO = lowest lethal dose tested
LD_{50} = the amount of a material, given all at once, which causes the death of 50% (one half) of a group of test animals.

Clearly, based on the table above, any consumption of ethanol as a residual substance on Xanthan gum will pose no threats to human health, such as those discussed above, since the estimated consumption is several orders of magnitude below that listed as toxic [Egland, *et al.*, 2001 Vol 2 Tab 25].

I. Ethanol Toxicity Compared to Other Alcohols

The three most common alcohol poisonings result from ethanol, methanol, and isopropanol. Recognition of the morbidity and mortality that may result from ingestion of small quantities of methanol because of its widespread use in non-food consumer products is particularly important for emergency medicine. Affected organs vary depending on the type of alcohol ingested [Church, *et al.*, 1997 Vol 2 Tab 13 and Albertson, 1999 Vol 2 Tab 2].

1. Ethanol

Ethanol (ethyl alcohol) is an aliphatic alcohol present in aftershaves, colognes, perfumes, mouthwashes, over-the-counter (OTC) medications, and a myriad of alcoholic beverages and food flavoring agents. In normal diets ethanol is constantly consumed, albeit generally in small quantities. Historically, consumption at this level has not been associated with adverse effects. However, there is no such history of chronic use in foods for either isopropanol or methanol.

Nonetheless, when consumed in large quantities, ethanol is a direct CNS depressant that causes decreased motor function and decreased consciousness level. At high concentrations, ethanol is an anesthetic and can cause autonomic dysfunction (e.g., hypothermia, hypotension), coma, and death from respiratory depression and cardiovascular collapse.

Ethanol is easily absorbed from the stomach and small intestine. When the stomach is empty, peak levels are reached 30-90 minutes after acute ingestion. Food present in the stomach delays absorption. Total absorption may take as long as six hours [Church, *et al.*, 1997 Vol 2 Tab 13 and Albertson, 1999 Vol 2 Tab 2].

Metabolism of ethanol is carried out in the liver by several enzymes, including alcohol dehydrogenase, aldehyde dehydrogenase, microsomal ethanoloxidizing system (MEOS) or CYP2E1, and peroxisomal catalase. MEOS accounts for about 5% but may increase to 25% in the chronic drinker. Normally, catalase makes a small contribution to ethanol metabolism; its role is more significant at high serum ethanol concentrations.

Nonhabituated persons metabolize ethanol at 13-25 mg/dUh. In persons with alcoholism, this rate increases to 30-50 mg/dUh. Metabolism rates vary greatly between individuals and cannot be predicted. Similarly, because of tolerance, blood alcohol concentrations (BACs) must be interpreted in conjunction with history and clinical presentation. Some individuals with chronic alcoholism may have an almost normal mental status and neurological examination, yet have BACs of 400 mg/dL. Conversely, nonhabituated drinkers may show marked effects of intoxication at very low BACs [Church, *et al.*, 1997 Vol 2 Tab 13 and Albertson, 1999 Vol 2 Tab 2].

2. Methanol

Methanol (methyl alcohol) is found in cleaning materials, solvents, paints, varnishes, Sterno fuel, formaldehyde solutions, antifreeze, gasohol, "moonshine," windshield washer fluid (30-40% methanol), and duplicating fluids.

Methanol is potentially toxic in amounts as small as a single mouthful. It is a central nervous system (CNS) depressant, and when metabolized by hepatic alcohol and aldehyde dehydrogenase, methanol forms formaldehyde and formic acid, both of which are toxic. The eyes, CNS, and GI tract are affected. Formic acid is the primary toxin that accounts for the majority of the metabolic acidosis, and ocular toxicity [Burkhart, *et al.*, 1990 Vol 2 Tab 9 and Albertson, 1999 Vol 2 Tab 2].

Formic acid inhibits cytochrome oxidase in the fundus of the eye. Disruption of the axoplasm is due to impaired mitochondrial function and decreased ATP production. Swelling of axons in the optic disc and edema result in visual impairment. Degradation of formic acid is folate dependent. Thus, if a folate deficient person ingests methanol, toxicity may be more severe due to the increased accumulation of formic acid.

Approximately 90-95% of methanol metabolism occurs in the liver, while 5-10% is excreted unchanged through the lungs and kidneys. Methanol is primarily metabolized by alcohol and aldehyde dehydrogenase. At low serum concentrations (<20 mg/dL) and during hemodialysis, methanol elimination is quick and first-order, with an elimination half-life of about 3 hours. At higher serum concentrations, methanol elimination is slow and zero-order, at 8.5 mg/dUh. Thus, following large doses, methanol is metabolized and eliminated very slowly. Formaldehyde has a short half-life, lasting only minutes. Formic acid is metabolized much more slowly, and it bioaccumulates with significant methanol ingestion.

3. Isopropanol

Isopropanol is found in OTC rubbing alcohol (70% isopropanol), antifreeze, skin lotions, and home cleaning products. As mentioned in the Section above, isopropanol is not chronically consumed in the course of a normal diet.

Death from ingestion of isopropanol is uncommon, however, isopropanol has 2-3 times the potency of ethanol and causes hypotension and CNS and respiratory depression more readily than ethanol. Peak levels occur approximately 30 minutes after ingestion because of rapid GI absorption, which is delayed in the presence of food. Serum levels more than 400 mg/dL are potentially fatal [Albertson, 1999 Vol 2 Tab 2, Smith, 1983 Vol 4 Tab 61 and Teta, *et al.*, 1992 Vol 4 Tab 68].

Approximately 20-50% of isopropanol is excreted unchanged by the kidney, while 50-80% is converted in the liver to acetone, which is a CNS depressant in its own right. Acetone is excreted primarily by the kidneys, with some excretion through the lungs. The elimination half-life of isopropanol is 4-6 hours; that of acetone is 16-20 hours. The prolonged CNS depression seen with isopropanol ingestion is partially related to acetone's CNS depressant effects [Albertson, 1999 Vol 2 Tab 2 and Smith, 1983 Vol 4 Tab 61].

Based on the comparative information presented above regarding the toxicities of three common alcohols, the sponsors submit that the use of ethanol rather than isopropanol for the extraction and purification of Xanthan gum, should pose no health threat to the public. Further, consumers are exposed to ethanol in the diet on a regular basis, and other foods, flavorings, and beverages are either extracted or prepared with ethanol without adverse affects; whereas there is no such record of regular (or repeated) exposure to low levels of isopropanol in the human diet. Therefore, ethanol may represent an alcohol for purification that has an even higher margin of safety than isopropanol, which is in common use in the gum industry today.

J. Other Approvals

The amount of various alcohol residuals remaining following the purification and recovery of Xanthan gum have been considered by various health authorities and regulatory bodies. Recently, the Joint Expert Committee on Food Additives (JECFA) determined that residual levels of either ethanol or isopropanol lower than 500 mg/kg, in conjunction with an unlimited Average Daily Intake (ADI) would pose no threat to the public, nor result in the toxicological effects of alcohol described above [JECFA, FNP Addendum 7, 1999 Vol 3 Tab 34].

This limit on residual alcohol for Xanthan gum is currently the lowest required by any of the major food codex standards. Using the maximum estimated mean intake, for Xanthan gum purified by recovery with ethanol established in Section II of this report (0.65 grams per day), and the maximum residual levels of ethanol (12 ppm) determined in Section I of this report, an intake of ethanolic residue from Xanthan gum purified by recovery with ethanol would equal 7.8 micrograms (0.0000078 g) per day, an insignificant amount. Data presented earlier in this report showed that the combined alcoholic residue found in several lots of ZZBP Xanthan gum ranged from non-detectable to 18 ppm. Therefore, the manufacture of Xanthan gum purified by recovery with ethanol yields residual alcohol levels that are below the JECFA limit by a factor of about 30.

K. Occupational Exposure to Xanthan Gum

Sargent, *et al.,* 1990, stated that until their publication, there was no body of literature pertaining to the occupational health hazards associated with handling xanthan gum [Sargent, *et al.,* 1990 Vol 4 Tab 59]. However, the authors described Xanthan gum as a nontoxic compound, particularly when consumed. Acute skin and eye irritation tests had indicated that the compound was nonirritating and was negative in an intracutaneous guinea pig sensitization test. However, Xanthan gum had been shown to induce proliferation of mouse B lymphocytes and thymocytes *in vitro* with concomitant antibody synthesis. Additionally, bulk Xanthan gum contains approximately 4-5% proteinaceous material derived from *X. campestris* which might contribute to allergic reactions in sensitive individuals [Sargent, *et al.,* 1990 Vol 4 Tab 59].

The authors reported that during the more than 20 years of Xanthan gum production to that time, there had been sporadic reports of "flu-like" symptoms in workers handling Xanthan gum powder. Although, information on the frequency, severity, and the duration of the symptoms had been generally anecdotal. The symptoms observed were variable, but most frequently had been described as chest tightness or heaviness, cough, and a self-limited flu-like response [Sargent, *et al.,* 1990 Vol 4 Tab 59].

Therefore, the research group performed a study to determine the relationship between the handling of Xanthan gum powder and reports of flu-like symptoms and/or hypersensitivity pneumonitis. Specifically, a medical evaluation was performed to determine the nature and prevalence of flu-like symptoms, whether or not subsymptomatic effects were evident that would be detectable by pulmonary function tests, and to examine for evidence of chronic pulmonary effects in workers exposed to Xanthan gum powder. In addition, a joint industrial hygiene survey was performed to determine the potential for exposure to Xanthan gum powder under normal operating conditions [Sargent, *et al.,* 1990 Vol 4 Tab 59].

All employees with potential exposure to Xanthan gum powder were identified and grouped according to the average percentage of time spent in the plant, not the expected intensity of exposure to Xanthan gum dust. The exposure groups were group A, exposed 60% to 100% of the time; group B, exposed 20% to 60% of the time, group C, exposed 1% to 20% of the time; and group D, exposed less than 1% of the time. Finally, the unexposed cohort, group E, was chosen from areas that were remote from the Xanthan gum manufacturing site. This group included employees from the personnel, purchasing, engineering, and administration departments whose structural environments were separate office-type buildings.

Immunological screening tests were considered, but were not included in the study protocol because preliminary tests to detect a specific antibody response to Xanthan gum, in one index case and in rabbits, were negative [Sargent, *et al.,* 1990 Vol 4 Tab 59].

At the time of publication there were no established exposure limits for Xanthan or for any other gum. The recommendation followed by the plant and throughout the industry was an 8-hour time-weighted average nuisance dust level of 10mg/m^3 respirable dust. This level was supported by the California Occupational Safety and Health Administration in response to a special study conducted between 1975 and 1977.

Plant workers were examined for changes in pulmonary functions over the first day of the workweek and for the entire workweek. Employees relating any symptom reported in a health-related questionnaire to a chemical exposure at work were classified as symptomatic for the purposes of this evaluation. All other plant employees were considered asymptomatic. No differences between the symptomatic group and the asymptomatic group were statistically significant. A further analysis was performed to determine whether any sex differences existed in the group reporting symptoms compared with those who did not. No statistically significant sex differences were found [Sargent, *et al.,* 1990 Vol 4 Tab 59].

The results of the questionnaire revealed that respiratory symptoms were reported by all exposure groups but that there was a slightly increased prevalence of nose and throat irritation and illness related to exposure to materials at the work site in group A. However, no significant acute changes in pulmonary function were found in any of the exposure groups. Of the four exposure groups, group B revealed slight decreases in pulmonary function over the first day of the workweek. These changes, however, were not statistically significant and may possibly be accounted for by the increased average age of the group.

Group A, which had the largest percentage of time in the milling area, demonstrated no significant change in pulmonary function over the first work day or over the week.

This study revealed that no acute or chronic effects on pulmonary function could be measured from Xanthan gum exposure. If acute effects were to be found, it was anticipated that they would be seen in workers in group A, which had the highest percentage of time spent in the millroom (60% to 100%), the highest average exposure to xanthan gum dust (0.1% to 1.9 mg/m^3), and a slightly increased prevalence of reported respiratory symptoms, even though they had been on the job the shortest period of time (13.5 months). However, no such acute effects were found. On the other hand, any chronic or long-term effects would have been expected in group B, whose members had worked for the longest period of time in the millroom, but by virtue of their jobs (now supervisory), spent less time during the day in the millroom. No chronic long-term effects were revealed in this group [Sargent, *et al.,* 1990 Vol 4 Tab 59].

Previous reports of flu-like symptoms generally followed abnormal events such as a baghouse fire or line failures, when operators were almost certainly exposed to transient, but unusually high Xanthan gum concentrations. Under current, normal operating procedures, no increase in reported symptoms and no acute or chronic effects on pulmonary function were evident, based on the study protocol. Therefore, the authors recommended that current standard operating procedures and control measures including the use of current nuisance dust standards should continue in the plant [Sargent, *et al.,* 1990 Vol 4 Tab 59].

L. Summary

Xanthan gum is a high molecular weight heteropolysaccharide produced by the partial fermentation using the non-pathogenic organism *Xanthomonas campestris*. Jansson *et al.*, described the primary structure of the Xanthan gum molecule as a backbone of 1,4-linked β-D-glucose with side chains containing two D- mannose and one D-glucuronic acid units [Jansson *et al.*, 1975 Vol 3 Tab 33].

As a polysaccharide, Xanthan gum meets the newly refined definition for dietary fiber, [Institute of Medicine Report, 2001 Vol 3 Tab 31]. The presence in the human intestinal system of a known mechanism for the partial fermentation of Xanthan gum strongly suggests that humans have the capacity to safely consume Xanthan gum at much greater concentrations than they encounter in foods products today without causing gastrointestinal upsets associated with consumption of excessively high levels of non- or partially digestible soluble dietary fiber [Rocks, 1971 Vol 4 Tab 56]. Xanthan gum contains D-glucose, D-mannose, and D- glucuronic acid as the dominant hexose units. Its structure is unusual, in that it contains 3.0-3.5 % pyruvic acid and about 6.3% acetyl [Sloneker *et al.*, 1962 Vol 4 Tab 60 and Stankowski *et al.*, 1993 Vol 4 Tab 62]. Chemical analyses have demonstrated that Xanthan gum purified by recovery with ethanol is identical in composition and structure to that purified with isopropanol. Therefore, its physiological effect will be no different from the Xanthan gum, which is currently, the article of commerce in the United States.

Woodard, *et al.*, 1973 published a review of the animal safety studies conducted earlier on Xanthan Gum [Woodard, *et al.*, 1973 Vol 4 Tab 81]. These included a two-year feeding study in rats and dogs and a three-generation reproduction study in rats. Neither treated rats or dogs who consumed from 0.0 to 1.0-g/kg b.w./day showed differences from control animals with respect to survival, food intake, body weight gain, electrocardiograms, blood pressures, hemograms, gross necropsy

observations, organ weights or histopathologic observations. A dose-related increase in fecal weight, an increase in the specific gravity of the urine and a more frequent presence of urinary albumin for dogs in the 1.0-g/kg b.w./day group was noted and could be the basis for setting a no-observed-adverse-effect-level (NOEL) for Xanthan gum [Woodard, *et al.*, 1973 Vol 4 Tab 81].

Eastwood, 1987 researched the dietary effects of Xanthan gum in humans. Male volunteers consumed, on each of 23 consecutive days, a weight of Xanthan gum equal to 10.4 g and 12.9 g per day. Measurements before and at the end of the test period showed that the ingestion of Xanthan gum, as a pre-hydrated gel, acted as a bulking agent in terms of its effects on fecal wet and dry weight and intestinal transit time, but had no significant effect on plasma biochemistry, hematological indices, urinalysis parameters, glucose tolerance and insulin tests, serum immunoglobulins, triglycerides, phospholipids and HDL cholesterol, breath hydrogen and breath methane concentrations [Eastwood, 1987 Vol 2 Tab 23].

The effects of feeding Xanthan gum on colonic function in man has been extensively studied [Daly, 1993 Vol 2 Tab 18]. Xanthan gum (15 g/d) was given for 10 days to eighteen normal volunteers. No untoward effects were noted. Xanthan gum was found to be a highly efficient laxative agent causing significant increases in stool output ($P < 0.01$), frequency of defecation ($P < 0.05$) and flatulence ($P < 0.01$), while having variable effects on transit time [Daly, 1993 Vol 2 Tab 18].

Xanthan gum has been studied as a dietary fiber in the management of diabetes mellitus. The feeding of Xanthan gum lowered fasting and postload serum glucose and reduced fasting levels of total plasma cholesterol in diabetic subjects. Subjects reported a sense of fullness after consuming twelve grams per day of Xanthan gum as an ingredient in muffins (two grams per muffin), but no severe digestive symptoms were noted [Osilesi, *et al*, 1985 Vol 3 Tab 48].

Occupational exposure to Xanthan gum dust has been studied, and no chronic or acute reduction in pulmonary function was observed for either new hires, or long-term employees. Reports of "flu-like" symptoms were found to have been associated with exacerbating events, such as fires or line breaks.

The following list indicates that toxic levels of ethanol far exceed any residual ethanol that may be encountered in the consumption of Xanthan gum purified by recovery with ethanol.

Ethanol Toxicity Data Compared to ZZBP Xanthan Gum Residuals

- 700 mg/kg oral-man TDLO
- 2000 mg/kg oral-child LDLO
- 50 mg/kg oral-man TDLO
- 1400 mg/kg oral-human LDLO
- 0.13 μg/kg for a 60 kg adult; Estimated mean daily intake of ethanol as a residual on Xanthan gum, as manufactured by ZZBP
- 0.26 μg/kg for a 60 kg adult; Estimated daily intake of ethanol as a residual on Xanthan gum (as manufactured by ZZBP), when consumed at the 90th percentile

The amount of various alcohol residuals remaining following the purification and recovery of Xanthan gum have been considered by various health authorities and regulatory bodies. Recently, the Joint Expert Committee on Food Additives (JECFA) placed the tightest restriction on residual alcohol levels of all major food regulatory bodies by lowering it to 500 mg/kg b.w. Combining a residual ethanol level with an unlimited Average Daily Intake (ADI) would pose no threat to the public, nor result in toxic effects from alcohol consumption [JECFA, FNP Addendum 7, 1999 Vol 3 Tab 34]. Using analytical data for the residual levels of ethanol (12 ppm) in Xanthan gum as manufactured by ZZBP, and the maximum estimated mean intake, (0.65 grams per day), the Sponsors have determined that consumption of ethanolic residue from Xanthan gum purified by recovery with ethanol would equal 7.8 micrograms (0.0000078 g) per day, an amount well below the maximum set by JECFA.

None of the animal or human studies reviewed in this report suggested any significant or consistent untoward effects attributable to the consumption of Xanthan gum in foods. Therefore, ISI and ZZBP submit that Xanthan gum purified by recovery with ethanol is a safe and suitable food ingredient, provided that it is used in accordance with current Good Manufacturing Practice.

Appendix I
Expert Opinions

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The Report of the Generally Recognized As Safe (GRAS) Status of Xanthan Gum (Purified by Recovery with Ethanol) By an Independent Expert GRAS Panel

The undersigned, an independent panel of recognized experts (Expert Panel), qualified by their scientific training and relevant national and international experience to evaluate the safety of food and food ingredients, was convened by Ingredients Solutions, Inc. (ISI) and Zibo Zhongxuan Biological Product Co, Ltd. (ZZBP) to determine the Generally Recognized As Safe (GRAS) status of Xanthan Gum, purified by recovery with ethanol, for use as a food ingredient.

A comprehensive search of the scientific literature for information on the safety/toxicity of Xanthan Gum, purified by recovery with ethanol, through April 2002 was undertaken by Lee Dexter and Associates and was made available to the Expert Panel. Additional information was provided by ISI and ZZBP. The members of the Expert Panel independently critically evaluated the information provided and other information deemed appropriate or necessary. Following this critical evaluation, the Expert Panel conferred by telephone and unanimously agreed that Xanthan Gum, purified by recovery with ethanol, is GRAS for use as a food ingredient when produced in accordance with current Good Manufacturing Practice (cGMP) and meeting the specifications described herein.

Xanthan Gum is an anionic bacterial polysaccharide composed of a beta - (1→4)-D-Glc (cellulosic) backbone with a trisaccharide side chain linked to the C3 of every second glucose residue. The side chain is beta-D-Man- (1→ 4)- beta-D-GlcA- (1→2)- alpha-D-Man- (1→3) with approximately 50% of the terminal D-mannose units containing a pyruvic acid moiety as a 4,6-cyclic acetal. The non-terminal D-mannose unit is stoichiometrically substituted at *O-6* with an acetyl group. Xanthan Gum has a molecular weight of about 2-4 million with a low polydispersity. The polymer hydrates completely, even in cold water.

Xanthan gum is an exopolysaccharide produced by fermentation using the non-pathogenic organism *Xanthomonas campestris*. The source organism is inactivated/destroyed during the thermal treatment phase that follows fermentation. The gum is isolated from the bulk medium by precipitation with ethanol. The precipitate is centrifuged and the fibers of Xanthan Gum are then dried, milled, sieved and packaged. All raw materials used in the manufacture of Xanthan Gum are food grade and all raw materials used in the fermentation process are steam sterilized. The sterilized media is then inoculated with *Xanthomonas campestris* and fermentation proceeds under agitation. Sterile air is piped into the fermentation vessels. Xanthan gum purified by recovery with ethanol is identical in chemical structure to that recovered with isopropanol.

Aqueous solutions of Xanthan exhibit a very high viscosity, even at low concentrations, and very strong pseudoplasticity with no evidence of thixotropy.

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Specifications for several product variants are given below in Table 1

Table 1: Food Grade Chemical Specifications for ZZBP Xanthan Gum Products

Product Type:	*ZB2*	*ZB3*	*ZBG*	*ZBT*
Appearance:	Creamy White, Free Flowing Powder			
pH:	6.0 - 8.0	6.0 - 8.0	6.0 - 8.0	6.0 - 8.0
Viscosity:	1300 - 1800 cPs	1300 - 1800 cPs	1300 - 1800 cPs	1300 - 1800 cPs
V1:V2	1.02 - 1.45	1.02 - 1.45	1.02 - 1.45	1.02 - 1.45
Purity				
Ash:	<13%	<13%	<13%	<13%
Moisture:	<13%	<13%	<13%	<13%
Pyruvic acid:	>1.5%	>1.5%	>1.5%	>1.5%
Nitrogen:	<1.5%	<1.5%	<1.5%	<1.5%
Ethanol	< 500 mg/kg	< 500 mg/kg	< 500 mg/kg	< 500 mg/kg
Lead:	<0.1 ppm	<0.1 ppm	<0.1 ppm	<0.1 ppm
Particle size:	100% through 80 mesh (180 microns)	100% through 60 mesh (250 microns)	Min. 97% through 60 mesh (250 microns)	100% through 60 mesh (250 microns)
	Min. 92% to pass through 200 mesh (75 microns)	Min. 95% to pass through 80 mesh (180 microns)	Max. 15% to pass through 200 mesh (75 microns)	Min. 95% to pass through 80 mesh (175microns)
Total plate count:	<2000/g	<2000/g	<2000/g	<2000/g
Yeast & mold:	<100/g	<100/g	<100/g	<100/g
Salmonella sp:	Absent in 25.0 g	Absent in 25.0 g	Absent in 25.0 g	Absent in 1.0 g
E.coli:	Absent in 25.0 g	Absent in 25.0 g	Absent in 25.0 g	Absent in 1.0 g
Coliforms	Absent in 1.0 g	Absent in 1.0 g	Absent in 1.0 g	Absent in 1.0 g
Staphylococcus aureus:	Absent in 0.5 g	Absent in 0.5 g	Absent in 0.5 g	Absent in 0.5g
Clostridium sp:	Absent in 1.0 g	Absent in 1.0 g	Absent in 1.0 g	Absent in 1.0 g
Streptococcus sp:	Absent in 0.5 g	Absent in 0.5 g	Absent in 0.5 g	Absent in 0.5g
Pseudomonas aeruginosa:	Absent in 0.5 g	Absent in 0.5 g	Absent in 0.5 g	Absent in 0.5g
Packaging	Packaging: Net 20 kg	cardboard drums or multi-ply paper bags: carton: net 20kg		

Xanthan Gum is a multiple-use direct food additive that has been in use for decades. Some of the approved uses (21 CFR 170.3 (o)) include emulsifier, flavor enhancer, formulation aid, humectant, nutritional supplement, stabilizer and thickener, synergist and texturizer. Xanthan Gum is used at levels of 0.05% and 0.3 % of the finished food product.

The current per capita intake of Xanthan Gum from all sources is 100 mg/day or 1.7 mg/kg bw/day. The maximum estimated mean daily intake, the EDI, (90th percentile, eaters only) is 0.65 gram/day or approximately 10 mg/kg bw/day, using CFSII, 1994-1996 intake data. Xanthan Gum purified by recovery with ethanol will be a replacement for Xanthan Gum purified by recovery with methanol, ethanol, or isopropanol.

Xanthan Gum has been reviewed by JECFA and was assigned an ADI not specified ("A term applicable to a food substance of very low toxicity which, on the basis of the available data (chemical, biochemical, toxicological, and other), the total dietary intake of the substance arising from its use at the levels necessary to achieve the desired effect [cGMP] and from its acceptable background in food does not, in the opinion of JECFA, represent a hazard to health. For that reason, and for reasons stated in the individual evaluations, the establishment of an acceptable daily intake expressed in numerical form is not deemed necessary." JECFA, 1994). Xanthan Gum has also been approved as a food additive at 21 CFR §172.695. Xanthan Gum also meets the newly refined definition for dietary fiber (IOM, 2001).

Published studies in animals and humans support the safety of Xanthan Gum. It is noted that most of the published information on the safety of Xanthan Gum was with material purified with isopropanol. However, the Xanthan Gum purified with ethanol is chemically identical with that purified with isopropanol; that is, it is substantially equivalent. Therefore, the safety of Xanthan Gum purified with ethanol has been established.

The Expert Panel independently and collectively critically evaluated information and data summarized in the GRAS Report. The Expert Panel unanimously concludes that Xanthan Gum, purified with ethanol, meeting the specifications described herein and produced and used in accordance with cGMP is Generally Recognized As Safe (GRAS) by scientific procedures for use as a food ingredient.

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ZZBP Raw data, 2001.	4	82

Appendix III
Production Process

Appendix III

Manufacturing of Xanthan Gum Purified by Recovery with Ethanol

Xanthan gum is an exopolysaccharide produced by fermentation of the organism *Xanthomonas campestris*. The gum is soluble in an aqueous culture medium, and is typically fermented in large fermentation vats of 50 to 300 m^3 [Harris, *et al.*, 1990 Vol 2 Tab 22]. The medium contains suitable carbon and nitrogen sources, along with appropriate minerals and buffers. Fermentation generally requires temperatures of approximately 30°C and agitation for several days. In the early stages of fermentation oxygenation of the broth is required to promote growth of the organism. However, as the culture matures, gum production diminishes the effects of agitation, and lessens mass transfer within the fermentation vat. In order to promote the continued production of Xanthan gum, as well as other polysaccharides produced by fermentation, special mixers have been designed to maintain aeration and patented [US Patent 6,110,731 Vol 4 Tab 67].

Following fermentation, the medium undergoes a thermal treatment, during which the source organism is destroyed/inactivated [Harris, *et al.*, 1990 Vol 2 Tab 22]. This allows the product to be produced in a near-sterile condition. The gum is typically isolated from the bulk medium via alcohol precipitation. The precipitate is then subjected to centrifugation, and the Xanthan fibers are then dried, milled, sieved, and packaged.

1. ZZBP Xanthan Gum Production Summary

ZZBP has provided a process flowchart, which depicts the company's process for Xanthan gum production (See Figure 9). The company assures the purity of the production strain in its laboratory, where cultures are grown on plates or slants, inoculated into test flasks, fermented in the presence of appropriate media, and selected based on their morphology and their ability to produce Xanthan gum in the desired yields. Selected cultures are then lyophilized and stored until needed.

The packaged product is then held in the company's warehouse, until released for sale by the Quality Assurance Group.

Figure 1 - Production Scheme for Xanthan Gum Purified by Recovery with Ethanol
As manufactured by ZZBP, Peoples Republic of China

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2. Critical Process Controls for Manufacturing

In addition to critical controls on the innoculum, ZZBP has designed 12 Critical Control Points into its process. Those Control Points are shown in Table 1. Each Critical Control Point contains information on the process stage that it governs, the hazard that the product would be subjected to, if no control were in place, the Critical Limits, the Control to be performed by the production staff, the frequency of the control action, who has responsibility for the control, the location of records regarding the control, and the corrective action that must be taken, as well as the group responsible.

The Critical Control Points cover receipt of raw materials, mixing, sterilization, fermentation, and precipitation, milling, sieving, and packaging. If remedies are not available within the process, the product may be either degraded to industrial Xanthan gum, or the batch may be destroyed.

HACCP PLAN for ZIBO ZHONGXUAN BIOLOGICAL PRODUCT CO.,LTD. (Cont'd)

HACCP PLAN for ZIBO ZHONGXUAN BIOLOGICAL PRODUCT CO.,LTD. (Cont'd)

	Process Stage	Hazard	Critical Limits	Control	Control Freq.	Control Responsibility/ Procedure	Record	Corrective Action & Responsibility
10	Packaging	Microbiological Contamination	Compliance with Zhongxun Product Specifications/QA	Clean before and after packing of each batch; Sterilized air is conveyed to the packing room through a sealed pipe Sanitary check-up	Twice Each batch	Prod.Operation ZX/QS.C.0922 – 1999	Plant Unit	Reject or degrade if contaminated
11	Packaging	Foreign body contamination	Packaging is new, in clean, dry and good condition without foreign material or particles represent	Physical inspection before packing	Per piece	Prod Operation ZX/QS.C.0914 – 1999	Plant Unit	Unsuitable packaging shall be rejected
12	Packaging	Risk of metal contamination	Metal detector must work efficiently and be in clean condition Sensitivity should be tested No metal detected in product	Check sensitivity of metal detector each batch Metal Detection after packing Sensitivity requirement specified in QA procedures	Per piece	Prod Operation Warehouse Operation QA ZX/QS.C.0921 - 1999	QA Plant Unit Warehouse	The entire batch of failed product quarantined and degraded industrial grade Investigation of the process to identify Metal Sources

3. Hygiene Rules For Employees and Outside Contractors

ZZBP has also provided guidelines for employee practice, which extend as well to outside contractors. Those guidelines are listed below.

- Smoking is prohibited on site inside the building
- Eating and drinking is prohibited in the production areas, laboratory and warehouses.
- Insecure costume or hand jewelry, including watches, earrings, dangling jewelry shall be removed.
- All persons having sores, lesions, boils, etc must report their condition before beginning work.
- Combing hair, spitting, scratching skin or nose, washing equipment in hand wash basins, coughing or sneezing without a clean handkerchief, long/dirty finger nails, and nail varnish are unacceptable in warehouse/production areas.
- Clean uniform or lab coats are required before entering production areas, warehouse and laboratory. Hair restraints, and clean gloves must be worn when applicable.
- Hands must be washed thoroughly with a sanitizing soap after visiting toilets.
- Glass or crockery are not permitted in warehouse area.
- Small cuts and scrapes must be covered with sterile bandages.
- Questions should be addressed to the Quality Assurance Department.

4. Analytical Methods

ZZBP has provided standardized analytical methods in accordance with 21 CFR 172.695 for the testing of its Xanthan gum products. These procedures assure that the product is tested consistently against specification parameters. Additional methods may be used internally to provide more detailed insight into the quality of the product. Both types of methods are shown below. A sample internal Certificate of Analysis follows the methods.

Specification Test Methods for Xanthan Gum Purified by Recovery with Ethanol

Manufactured by: Zibo Zhongxuan Biological Product Co., Ltd

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1. **Appearance:**
   Inspect visually.

2. **Viscosity:**
2.1 Prepare a 1% salt solution of Xanthan gum in distilled water
2.2 Mix the solution until the substance is completely dissolved.
2.3 After 2 hours, measure the viscosity using an LV model Brookfield viscometer at 60 rpm with a No.3 spindle.

3. **pH:**
   Determine the pH by testing the solution as prepared above using a pH meter.

4. **Moisture:**
4.1 Measure 10 grams Xanthan gum.
4.2 Dry in a tared watch glass in an oven at 105±1°C for 2 hours.
4.3 Cool in a desiccator and reweigh.

5. **Ash:**
5.1 Measure accurately about 3g of Xanthan gum. Dry at 105°C for 4 hrs in a tared crucible, and
5.2 Incinerate at about 650°C until free from carbon.
5.3 Cool the crucible and contents in a desiccator, and weigh the ash.

6. **Particle Size:**
6.1 Measure accurately 10 grams of Xanthan gum.
6.2 Shake on a 60 mesh, 80 mesh and 200 mesh sieve with a shaker.

7. **V1:V2:**
   Viscosity at 23.9 °C determined as in 2 above / viscosity at 65.4°C

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8. **Pyruvic Acid:**

8.1 Dissolve 600mg Xanthan gum in water and bring to a total volume of 100ml.

8.2 Transfer 10ml of the solution into a flask and add 20 ml of 1 *N* hydrochloric acid.

8.3 Weigh the flask.

8.4 Reflux for 3 hrs.

8.5 Add 1 ml of a 1 in 200 solution of 2,4-dinitrophenylhydrazine in 2*N* hydrochloric acid into a 30 ml separatory funnel, then add 2 ml of the sample solution and mix.

8.6 Allow the solution to stand at room temperature for 5 min.

8.7 Extract the mixture with 5 ml of ethyl acetate.

8.8 Discard the aqueous layer.

8.9 Extract the hydrazone from the aqueous layer with three 5-ml portions of 10% sodium carbonate solution, collecting the extracts into a 50-ml flask. Dilute to volume with 10% sodium carbonate and mix.

8.10 Standard preparation: measure 45mg pyruvic acid and transfer into a 500 ml flask. Dilute to volume with water. Transfer 10 ml of this solution into a 50 ml glass-stoppered flask and continue as directed under sample preparation (step 8.2), beginning with adding 20 ml of 1*N* hydrochloric acid into the flask

8.11 Procedure: determine the absorbance of each solution with a suitable spectrophotometer in 1-cm cells at a maximum of about 375 nm, using 10% sodium carbonate as the blank. The absorbance of the "sample preparation" should be equal to or greater than that of the "standard preparation"

9. **Lead, Arsenic and Total Heavy Metals:**
Follow the procedure given in the current (4th) edition of Food Chemical Codex

10. **Microbiological limits:**

10.1 For yeast and mold, follow the procedure as given for microbial limit tests in the current (2000) edition of the U.S. Food and Drug Administration's Bacteriological Analytical Manual for Foods.

10.2 For total plate count of *E. coli* and *Salmonella* follow the procedures as given for microbial limit tests in the current (2000) edition of the United States Pharmacopoeia.

5. Recall and Tracking Program

a. Lot Numbers

ZZBP's manufacturing process is organized batchwise into a series of fermentation and purification steps. Each batch produced is assigned a unique lot number. Each finished lot is assigned a 5-digit number. The first digit equates to the plant at which the product was produced. The next two digits equate to the year of production, e.g., 01, for 2001, and the last three digits represent the production sequence. These three digits are a consecutive assignment from a serial numbering log. In this log, numbers are assigned consecutively by date, and therefore the date of production can be confirmed by the ZZBP Quality Assurance Group.

The reference numbers and quantities of all materials and processing aids used in each unit of fermentation and purification are also recorded for various lot numbers. Thus, it is possible for ZZBP to track not only the finished product, but also each raw material component that was used in the processing of that finished product. All finished products can be traced to a given two-day production period.

Retained samples for each lot are maintained for 2 years post expiration date or 4 years past the manufacturing date if no expiration date is assigned. All sales of ZZBP Xanthan gum are recorded and kept in secure files, and duplicate copies of records are stored at a location separate from the production facility. Thus each lot may be tracked to a final destination, and recalled if a foodborne emergency should arise.

b. Investigation Procedure

In the event of a complaint or foodborne illness resulting from use of the company's Xanthan gum product, a written policy guides an investigation procedure. This procedure is listed below, along with guidelines for management follow-up. The company has also designed various report forms to aid its quality investigation. These records are maintained in the company files.

Investigation Analysis:

- The sales department will submit a detailed report on the form: "Disposal Sheet of Product Recall".
- Related departments should investigate and analyze the problems mentioned on the form, and produce a detailed, written report that highlights the causes of these problems.
- Following a review of the results of the investigation analysis, related departments will draft a disposal plan and submit it to the Managing Representative for approval. The Managing Representative is then responsible for putting the disposal plan into effect.
- If the customer is not satisfied with the disposal, this should be relayed to the Managing Representative and General Manager.

Management Follow Up:

- The sales department will settle problems caused by product quality or food safety and take corrective action. These actions should be followed up, certified and evaluated. Effective actions should be recorded. If the corrective actions are not effective, new steps should be taken and the results should be followed up and certified until the customers are satisfied.
- Any amendment of this document should be executed according to "Control Procedure of Documents and Data".
- Both the Sales Department and other related departments are responsible for keeping records.

Quality Control Records Involved:

- Investigation Report of Customer's Complaint
- Response Record of Market Information
- Disposal Report of Production Recycle

Appendix IV
Food Categories

Appendix IV: Xanthan Gum (Purified by Recovery with Ethanol)

Table 1
GRAS Food Additive Categories and Sub-Categories

Food Category Code	GRAS Food Additive Categories	Realistic Levels of Xanthan Gum (%)
LIQ	All liquid foods, except water	
SOL	All solid foods, except salt & pepper	
TOT	Total - all foods, except water, salt & pepper	
01 Category	Baked goods, baking mixes	
01A	Bread and rolls, white	0.05
01B	Bread and rolls, dark	0.05
01C	Bread, other, incl. English muffins, bagels, onion rolls, poppy seed rolls, sesame seed rolls, etc.	0.05
01D	Pies, fruit-type (see 08I for fillings sold separately)	0.1
01E	Pies, custard-or pudding-type (see 20F for fillings sold separately)	0.1
01F	Pie crusts, pizza crusts, other crusts	0
01G	Cakes (see 17C for frostings sold separately)	0.1
01H	Cracker, unflavored; bread sticks, pretzels (see also 22 I and K)	0
01 I	Sweet rolls, doughnuts, coffee cakes	0.05
01J	Pancakes, waffles, French toast	0.2
01K	Quick Breads, incl. biscuits, muffins, batter bread (non-yeast leavened)	0.2
01L	Cookies, graham crackers	0.2
01M	Bread products, incl. crumbs, stuffings, batter dips, breadings, cubes (flavored & unflavored)	0.05
01N	Dietetic products, incl. bar-type foods (i.e. granola bars)	0.1
01O	Ice cream cones; fruit cake (including candied fruit)	0
01P	Other baked goods, baking mixes	0.2

Table 1, (continued)

Food Category Code	GRAS Food Additive Categories	Realistic Levels of Xanthan Gum (%)
02 Category	Breakfast Cereals	
02A	Ready-to-eat, presweetened cereal (25% or more sugar)	0
02B	Ready-to eat-, non-presweetened cereal (less than 25% sugar)	0
02C	High-nutrition cereal of any type (if fortified to 45% or more of the USRDA with one or more constituent)	0
02D	Hot, instant cereal	0
02E	Flavored oats, hot cooked, regular and quick	0
02F	Unflavored oats, hot cooked, regular, and quick	0
02G	Other cereals, hot cooked, regular, and quick	0
02H	Ready-to-eat so called "natural" cereal, regardless of sweetening level	0
02I	Dietetic cereal products, unused in GRAS/MC	0
02J	Other cereals, unused in GRAS/MC	0
03 Category	Other grain products, pastas	
03A	Plain pastas	0
03B	High-nutrition type pastas, unused in GRAS/MC	0
03C	Rice, flavored	0
03D	Rice, regular	0
03E	Flour	0
03F	Dietetic grain products, pastas	0
03G	Pasta/ rice/ noodles with sauce/ meat (except cheese sauce)-plain and Italian dishes	0.075
03H	Pasta/ rice/ noodles with cheese sauce	0
03I	Other grain products, pastas, (including barley, grits, corn meal, bulgar, etc.)	0

Table 1, (continued)

Food Category Code	GRAS Food Additive Categories	Realistic Levels of Xanthan Gum (%)
04 Category	Fats and oils	
04A	Margarine, all vegetable	0
04B	Butter (regular)	0
04C	Mayonnaise and salad dressing, standardized federal standards of identity)	0
04D	Other dressings, not standardized	0.2
04E	Salad and cooking oils, retail	
04F	Shortening, vegetable, retail	0
04G	Shortening, animal, retail or animal plus vegetable (including lard)	0
04H	Institutional-commercial baking fats, vegetable (shortening)	0
04I	Institutional-commercial baking fats, animal or animal and vegetable (shortening)	0
04J	Institutional-commercial frying fats, vegetable	0
04K	Institutional-commercial frying fats, animal or animal and vegetable	0
04L	Institutional-commercial hard butter, butter oil	0
04M	Dietetic fat and oil products (incl. low calorie salad dressings and sandwich spreads, diet margarine)	0.3
04N	Other fats and oils (incl. butter/margarine blend)	0
04O	No fat salad dressing and mayonnaise	0.5
05 Category	Milk, milk products	
05A	Whole milk	0
05B	Chocolate-flavored milk and dairy based drinks (but not incl. diet beverages) see 05J	0.2
05C	Non-chocolate flavored milks and dairy based drinks	0.2
05D	Eggnog	0.2

Table 1, (continued)

Food Category Code	GRAS Food Additive Categories	Realistic Levels of Xanthan Gum (%)
05E	Yogurt	0.2
05F	Sour cream, sour cream dips	0.15
05G	Evaporated milk, evaporated skim milk	
O5H	Creams (not incl. non-dairy types see 28B and C)	0.2
05I	Whey, caseinates, other dairy-derived products	0.1
05J	Dietetic milk products (incl. meat replacement or low calorie cocoa and flavored milk)	0.2
05K	Skim/ low-fat milk	
05L	Buttermilk	0.1
05M	Other milk, milk products (incl. sweetened condensed milk)	
05N	Milk dry or powdered, no reconstituted	
05O	Dietetic beverage powders, no reconstituted (meal replacement or low calorie cocoa/flavored milk)	0.1
05P	Beverage powders, not reconstituted (cocoa mixes and flavored milk drinks—see 05B & 05C for fluid)	0.15
06 Category	Cheese	
06A	Natural cheese, except grated or shredded	0
06B	Natural cheese, purchased as grated or shredded	0
06C	Processed cheese, cheese food, spread, fondue	0.2
06D	Cottage cheese and similar types	0.1
06E	Cream cheese, cheese dip, neufchatel products	0.2
06F	Dehydrated cheese	0
06G	Dietetic cheese products(low calorie, low fat, low sodium)	0.1
06H	Other cheese products (i.e. cheese mixture or combinations)	0

Table 1, (continued)

Food Category Code	GRAS Food Additive Categories	Realistic Levels of Xanthan Gum (%)
07 Category	Frozen dairy desserts	
07A	Ice cream, ice milk, vanilla-type flavors only (including novelties)	0.1
07B	Ice cream, ice milk, chocolate-type flavors only (including novelties)	0.1
07C	Ice cream, ice milk, other-type flavors only (not chocolate or vanilla, including novelties)	0.2
07D	Sherbets	0.2
07E	Mellorine	0.2
07F	Milk Shakes, malted milks	0.15
07G	Dietetic frozen dairy dessert products	0.2
07H	Other frozen dairy desserts	0.2
08 Category	Fruits and Fruit Juices	
08A	Fruits, fresh	0
08B	Fruits, canned	0
08C	Fruits, frozen	0
08D	Fruits, dried	0
08E	Fruits, semi-moist, unused in GRAS/MC	0
08F	Juices, natural, canned, or bottled	0.05
08G	Juices, natural, frozen	0
08H	Drinks and ades containing 10% or more of natural juice (see 23A)	0.05
08I	Fruit-type pie fillings	0.1
08J	Dietetic fruits and fruit juices (incl. low calorie)	0.05
08K	Maraschino fruits	0
08L	Other fruits and fruit juices (including pickled, fruit salads, etc.)	0

Appendix IV, (continued)

Table 1, (continued)

Food Category Code	GRAS Food Additive Categories	Realistic Levels of Xanthan Gum (%)
09 Category	Fruit ices, water ices	
09A	Cup, stick, or other type ices (including "popsicle" types)	0.1
09B	Unused in GRAS/MC	
10 Category	Meat, meat products	
10A	Meats, fresh or frozen, plain, including ground meat (beef, pork, veal, lamb, etc.)	0
10B	Meats, fresh or frozen, prebreaded, commercial	0
10C	Sausage, smoked/cooked, including bologna, links, polish, weiners, franks, knockwurst)	0.4
10D	Sausage, fresh and precooked pork, including bratwurst, ground patties, etc.	0.4
10E	Sausage, specialty (dry, ethnic, fermented, etc. including summer sausage, Italian, salami, Thuringer	0
10F	Luncheon meats, processed	0.4
10G	Meat products, fabricated, with or without extenders, sectioned and formed, fresh, frozen, or cooked	0.4
10H	Unused in GRAS/MC	
10I	Unused in GRAS/MC	
10J	Beef products, cured, including chopped beef, pastrami, and beef bacon	0
10K	Ham, cured, processed, etc. incl. smoked and pre-cured	0.1
10L	Bacon	0
10M	Dietetic meat products (spreads and salads only)	0.1
10N	Seasoned meat; meat with sauce/vegetables	0.15
10O	Meat with pasta/rice; hash, spreads and salads	0.15
10P	Other meat products	0

Table 1, (continued)

Food Category Code	GRAS Food Additive Categories	Realistic Levels of Xanthan Gum (%)
11 Category	Poultry, poultry products	
11A	Fresh, plain chicken, turkey, game birds, organs	0
11B	Canned poultry	0.2
11C	Self-basting turkey, commercial frozen	0.1
11D	Frozen, commercial breaded, raw or cooked poultry	0
11E	Frozen, commercial unbreaded, raw or cooked poultry	0
11F	Fresh, commercial breaded or batter dip poultry	0
11G	Poultry rolls, processed	0.2
11H	Frozen, raw, prestuffed poultry	0
11I	Dietetic poultry products (spread and salads only)	0.1
11J	Poultry with sauce and/or vegetables	0.05
11K	Other poultry products	0
12 Category	Eggs, egg products	
12A	Fresh eggs	0
12B	Dried whole eggs, powdered	0
12C	Omelet mixes	0
12D	Frozen whole eggs	0
12E	Dried egg yolks	0
12F	Dried egg whites (including meringue mix)	0
12G	Modified eggs, egg substitutes (including dietetic products)	0
12H	Other egg products (including egg salad, ready-to-use, boil-in-pouch types, etc.)	0
13 Category	Fish, seafood	
13A	Fish, not processed, fresh or frozen, not commercial breaded, stuffed, or cooked (steaks, filets, etc.)	0
13B	Fish, processed, frozen (including commercial breaded, stuffed, cooked, etc.	0.1

Appendix IV, (continued)

Table 1, (continued)

Food Category Code	GRAS Food Additive Categories	Realistic Levels of Xanthan Gum (%)
13C	Fish, processed, not frozen	0.1
13D	Shellfish (crustacean), not processed, fresh or frozen	0
13E	Shellfish (crustacean), not processed, fresh or frozen, not commercial breaded, stuffed, or cooked	0.1
13F	Shellfish (mollusks), not processed, fresh or frozen	0
13G	Shellfish (mollusks), processed, commercially breaded, stuffed, or cooked, etc.	0.1
13H	Fish or shellfish, canned, without seasoning, i.e. no mustard, ketchup, etc.	0.1
13I	Fish or shellfish, smoked	0
13J	Fish or shellfish, semi-cured (e.g. pickled or salted)	0
13K	Fish or shellfish, fabricated, frozen (balls, bits, cakes, loafs, etc. including all fish sticks)	0.1
13L	Dietetic seafood products (spreads and salads only) and /or vegetables	0.1
13M	Shrimp cocktail	0
14 Category	Vegetables, vegetable juices	
14A	Vegetables, fresh	0
14B	Vegetables, canned (including sweet potatoes and yams but excluding white potatoes, see 14E)	0
14C	Vegetables, frozen, plain without sauces	0
14D	Vegetables, dried	0
14E	Potatoes, white, canned	0
14F	Potatoes, white, frozen	0
14G	Potatoes, white, dried	0
14H	Vegetables, specialty, canned or frozen, including all with sauce or meat (boil in bag, oriental, mexicorn, etc.)	0.05
14I	Vegetable juices	0.05
14J	Dietetic vegetable products	0

Appendix IV, (continued)

Table 1, (continued)

Food Category Code	GRAS Food Additive Categories	Realistic Levels of Xanthan Gum (%)
14K	Cream style corn	0
14L	Baked beans	0
14M	Potato casseroles	0
14N	Other vegetable products (including ready to eat specialty dishes)	0
15 Category	Condiment, relishes	
15A	Olives	0
15B	Pickles, relishes (chopped pickle type)	0.3
15C	Mustard, prepared	0.3
15D	Catsup	0.4
15E	Condimental sauces including chili sauce, chutney, "A-1" types, soy sauce	0.3
15F	Dietetic condiments, relishes	0.3
15G	Other condiments, relishes	0
16 Category	Candy	
16A	Chocolate, plain, including baking chips	0
16B	Chocolate with fruit, nuts, and/or cereals	0
16C	Chocolate covered, plus other ingredients including panned and soft centers	0
16D	Sugar-panned candies, not jelly beans, "M&M" types chocolate centers	0
16E	Cough drops, candy-type	0
16F	Hard candies, including sucker types and pressed tablets	0
16G	Soft candy, marshmallow nougat types	0.1
16H	Soft candy, toffee, caramels, other	0.1
16I	Gum types and jellies including jelly beans	0.1
16J	Dietetic candy products	0
16K	Other candy products including non-chocolate baking chips	0

Table 1, (continued)

Food Category Code	GRAS Food Additive Categories	Realistic Levels of Xanthan Gum (%)
17 Category	Sugars, frostings, etc	0.05
17A	Sugar, granulated, white (sucrose)	0
17B	Other sugars, including raw, brown, powdered, confectioners, dextrose, fructose	0
17C	Frostings, icings, glazes	0.1
17D	Corn syrup, solids, bulk, commercial (see also 19F)	0
17E	Other sugars, frostings (including pellets, cakes decorations)	0
18 Category	Jams, Jellies, Sweet Spreads	0.3
18A	Jams, preserves, marmalades, butters	0.3
18B	Jellies, unflavored	0.3
18C	Jellies, flavored	0.3
18D	Dietetic jams, jellies, sweet spreads	0.3
18E	Other jams, jellies, sweet spreads (including sweet spreads, preserves, fruit spreads, etc.)	0.3
19 Category	Sweet sauces, toppings, syrups	0.1
19A	Sweet sauces and toppings, fruit types	0.1
19B	Sweet sauces and toppings, chocolate types	0.4
19C	Other sweet sauces and toppings (including nut toppings)	0.4
19D	Syrup, chocolate	0.4
19E	Syrup, maple and maple flavored	0
19F	Corn syrup, retail (See also 17D)	0
19G	Dietetic sweet sauces, toppings, syrups	0.4
19H	Other sweet sauces, toppings, syrups (including sweet meat glazes)	0.4

Table 1, (continued)

Food Category Code	GRAS Food Additive Categories	Realistic Levels of Xanthan Gum (%)
20 Category	Gelatins, puddings, custards	0.1
20A	Gelatin products	0
20B	Puddings, canned	0.1
20C	Puddings, dried to be cooked	0
20D	Puddings, dried, instant	0.1
20E	Custards	0.1
20F	Pie fillings, custard, or pudding type	0.1
20G	Dietetic gelatins, puddings, custards	0.1
20H	Other gelatins, puddings, custards	0.1
21 Category	Soups, soups mixes	
21A	Tomato base soups, canned or frozen (i.e. all tomato)	0
21B	Creamed or thickened soups, canned or frozen	0.2
21C	Other soups, canned or frozen	0
21D	Dry soups mixes	0.15
21E	Bouillon cubes, granules	0
21F	Dietetic soups, soup mixes	0.15
21G	Other soups, soup mixes	0
22 Category	Snack Food	0
22A	Snacks, fabricated and flavored, including curls, puffs, and extruded, corn chips, excluding potato chips and sticks 22C-22E	0.05
22B	Snacks, fabricated and unflavored (See 22A)	0
22C	Potato chips and sticks, fabricated, unflavored (eg. Chipos, Munchos, Pringles)	0
22D	Potato chips and sticks, not fabricated flavored	0.05
22E	Potato chips and sticks, plain	0
22F	Popped corn, flavored	0.05
22G	Popped corn, unflavored	0

Table 1, (continued)

Food Category Code	GRAS Food Additive Categories	Realistic Levels of Xanthan Gum (%)
22H	Appetizers, multi-components	0
22I	Snack crackers, filled	0
22J	Dietetic snack products	0
22K	Snack Crackers	0
22L	Other snack foods (including "Ritz" and "Hi-Ho" types	0
23 Category	Beverages Type I (Non-Alcoholic)	
23A	Beverages, uncarbonated, containing less than 10% of natural juices (see also 08H)	0.05
23B	Dry mixes (e.g. Tang, Wylers)	0.5
23C	Soft drinks, carbonated, cola-type	0
23D	Soft drinks, carbonated, fruit flavors	0
23E	Soft drinks, carbonated, other (including ginger ale, root beer, etc.)	0
23F	Carbonated water, plain soda	0
23G	Quinine water	0
23H	High-electrolyte and isotonic drinks (eg. Gatorade)	0.05
23I	Dietetic beverages, non-colas (including low calorie and sugar free drinks)	0.05
23J	Dietetic beverages, colas (including low calorie and sugar free drinks)	0
23K	Other beverages, non-alcoholic	0
23L	Plain bottled water	0
24 Category	Beverages Type II (Alcoholic)	
24A	Beer, ale, other malt beverages, including malt liqueur	0
24B	Brandy, whiskey, rum, vodka	0
24C	Gin	0
24D	Cocktails	0

Appendix IV, (continued)

Table 1, (continued)

Food Category Code	GRAS Food Additive Categories	Realistic Levels of Xanthan Gum (%)
24E	Vermouth, other flavored wines and aperitifs (see 24K for pop wines)	0
24F	Wine, table (14% alcohol or less)	0
24G	Wine, fortified (more than 14% alcohol)	0
24H	Wine, sparkling	0
24I	Wine, cooking	0
24J	Cordials, liqueurs	0
24K	"POP" wines, fruit wines	0
24L	Unused in GRAS/MC	0
25 Category	Nuts, nut products	0
25A	Peanut butter	0
25B	Peanut butter, imitation or whipped	0
25C	Peanut butter, commercial	0
25D	Peanut butter, imitation, commercial	0
25E	Nut spreads, including flavored spreads, mixtures with jams, marshmallows, etc.	0
25F	Nuts and seeds, dry roasted, and products thereof (other than 25A-25E), excluding candy	0
25G	Nuts and seeds, oil roasted	0
25H	Coconuts and coconut mixtures, excluding candy	0
25I	Dietetic nuts, nut products	0
25J	Candy covered nuts	0.05
25K	Other nuts, nut products (incl. unroasted nuts)	0
26 Category	Reconstituted vegetable proteins, analogs extenders	0.1
26A	Meat extenders incl. soy granules and powders	0.1
26B	Protein product analogs (meat, bacon, fish, poultry, cheese, etc.)	0.1
26C	Dietetic proteins, analogs, extenders	0.1

Table 1, (continued)

Food Category Code	GRAS Food Additive Categories	Realistic Levels of Xanthan Gum (%)
26D	Other proteins, analogs, extenders	0.1
26E	Protein product analogs—bacon bits only	0
26F	Protein product analogs—except bacon bits	0
27 Category	Gravies, sauces (non-condiment types)	0.15
27A	Meat type gravies and sauces, including "au-jus" brown, giblet, and poultry gravies, etc.	0.15
27B	Tomato base gravies and sauces, including barbeque, pizza, and spaghetti sauces; sloppy joe types (see also subcategory C)	0.5
27C	Tomato sauce, pasta, puree	0.4
27D	Milk base gravies and sauces, including cream types ("a la king", beamaise, etc.) cheese, sour cram, and white sauce	0.4
27E	Soup having major use as gravies and sauces	0.15
27F	Dietetics gravies and sauces	0.15
27G	Other gravies and sauces (including mushroom, onions, lemon, and vinegar based marinades and bastings)	0.5
28 Category	Dairy Product Analogs	0
28A	Filled Milk	0
28B	Toppings, creams, including imitation sour cream	0.2
28C	Coffee Whiteners	0
28D	Other dairy product analogs	0.05
28E	Coffee whiteners, powdered	0
28F	Coffee whiteners, liquid	0.05
31 Category	Chewing Gum	0
31A	Slab type chewing gum, fruit flavors	0
31B	Slab type chewing gum, mint flavors	0
31C	Slab type chewing gum, other flavors	0

Table 1, (continued)

Food Category Code	GRAS Food Additive Categories	Realistic Levels of Xanthan Gum (%)
31D	Candy coated gum, fruit flavors	0
31E	Candy coated gum, mint flavors	0
31F	Candy coated gum, other flavors	0
31G	Sugarless chewing gum	0
31H	Bubble gum all types, including slab and candy coated	0
31I	Unused in GRAS/MC	0
33 Category	Sugar substitutes	0
33A	Powder type sugar substitutes	0
33B	Tablet type sugar substitutes	0
33C	Liquid type sugar substitutes	0
33D	Other sugar substitutes	0
33E	Aspartame (Equal), packets	0
33F	Aspartame (Equal), tablets	0
45 Category	Main dishes, N.E.C.	0
45A	Pizza	0.1
45B	Main dishes, Mexican including tortillas, chili...)	0
45C	Main dishes, Chinese	0
45D	Large ground beef sandwiches with cheese (>200gm.)	0
45E	Large ground beef sandwiches without cheese (>200gm.)	0
45F	Regular ground beef sandwiches with cheese (< or = 200 gm.)	0
45G	Regular ground beef sandwiches without cheese (< or = 200 gm.)	0
45H	Other beef sandwiches	0
45I	Chicken/other poultry sandwiches	0
45J	Ham/pork/other plain meat sandwiches	0
45K	Fish/other seafood sandwiches	0
45L	Other meat sandwiches (sausage, lunchmeat, frankfurters, etc.)	0.05

Appendix IV, (continued)

Table 1, (continued)

Food Category Code	GRAS Food Additive Categories	Realistic Levels of Xanthan Gum (%)
45M	Other non-meat sandwiches with cheese	0
45N	Other non-meat sandwiches without cheese	0
46 Category	Food Coloring	0
46A	Food Coloring	0
47 Category	Dietary Supplements	1.5-4.5
47A	Dietary supplements, vitamins, etc.	0
48 Category	Seasonings and Flavorings	
48A	Products predominantly MSG (monosodium glutamate)	0
48B	Dry or powder seasonings and flavorings other than MSG (monosodium glutamate)	0.2
48C	Meat tenderizers	0
48D	Table salt, retail and institutional	0
48E	Unused in GRAS/MC	0
48F	Other seasonings and flavorings	0.2
49 Category	Coffee and Tea	0
49A	Coffee to be brewed, regular	0
49B	Coffee to be brewed, decaffeinated	0
49C	Coffee, instant, regular	0
49D	Coffee, instant, flavored	0.05
49E	Coffee, instant, decaffeinated	0
49F	Tea, not flavored, processed, ready to drink (e.g. canned, instant)	0
49G	Tea, flavored	0.05
49H	Other coffee and tea (including concentrates and postum types; tea bags)	0

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Table 1, (continued)

Food Category Code	GRAS Food Additive Categories	Realistic Levels of Xanthan Gum (%)
81 Category	Baby food-baked goods	0
81A	Baby food-all baked goods, incl. teething biscuits, baked finger foods, etc.	0
82 Category	Baby food-cereals	0
82A	Baby food-all cereals	0
83 Category	Baby food-formula products	0
83A	Baby food-prepared base formulas	0
84 Category	Baby food-processed fruit, juice, drink	0
84A	Baby food-ready to east processed fruit, juice, drinks (excluding apple sauce)	0
84B	Baby food-applesauce	0
85 Category	Baby food-meat products	0
85A	Baby food-ready to eat meat products, including strained, chopped, or cubed	0
86 Category	Baby food-poultry products	0
86A	Baby food-ready to eat poultry products and products mainly poultry	0
87 Category	Baby food-egg products	0
87A	Baby food-ready to eat egg products and products mainly egg	0
88 Category	Baby food-fish, seafood products	0
88A	Baby food-ready to eat fish, seafood products and products mainly fish, seafood	0

Table 1, (continued)

Food Category Code	GRAS Food Additive Categories	Realistic Levels of Xanthan Gum (%)
89 Category	Baby food-processed vegetables	0
89A	Baby food-ready to eat processed vegetables, including strained and chopped	0
90 Category	Baby food-pudding, custards, desserts	0
90A	Baby food-puddings and custards, ready to eat	0
90B	Baby food-other desserts, ready to eat (excluding puddings and custards)	0
91 Category	Baby food-soups	0
91A	Baby food-ready to eat soups	0
92 Category	Baby food-high meat dinners, cheese food	0
92A	Baby food-ready to eat high meat dinners, cheese food	0
93 Category	Baby food-combination dinners (meat and vegetable)	0
93A	Baby food-ready to eat combination dinners (meat and vegetable)	0
94 Category	Other and unidentified baby food	0
94A	Other and unidentified baby food	0

End Submission

AM

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MEMORANDUM

TO: DR. JEREMIAH FASANO, CFSAN

FROM: LEE DEXTER

DATE: APRIL 15, 2003

RE: CLARIFICATION TO AMENDMENT #1 OF GRN 121

Dear Dr. Fasano:

As we discussed by telephone, I would like to provide you with written clarification for one point presented in Amendment #1 to GRAS Notice 121. This point deals with the use of Xanthan gum purified by recovery with ethanol in dietary supplements.

(b) (4)

As a practical matter, Xanthan gum purified by recovery with ethanol has a relatively low self-limiting level of use. Most uses for xanthan gum in general are less than 0.5% (See Section III of GRN 121). Taking the example we discussed of a muffin, which has a serving size of 50 grams, the addition of Xanthan gum purified by recovery with ethanol to improve texture would add only 0.25 grams of fiber to the finished product. This would not be enough to justify a claim. Therefore, unlike wheat bran, which could be added to foods to substantially increase fiber content, the sponsors of GRN 121 did not intend that Xanthan gum purified by recovery with ethanol would be added as a fiber supplement to foods in general.

Dr. Fasano, if you require further explanation, please feel free to call me at the number listed above.