

GR



B

000249

**Generally Recognized As Safe (GRAS) Determination  
for the Addition of Polydextrose to Infant Formula as  
a Prebiotic Ingredient In Combination with  
Galactooligosaccharides**

August 2007

## Table of Contents

<b>I. Description of Substance</b>	<b>3</b>
<b>A. Identity</b>	<b>3</b>
1. Common or Usual Name	3
2. Chemical Names	3
3. CAS Registry Number	3
4. Composition	3
a) Molecular Formula	3
b) Molecular Weight	3
c) Degree of Polymerization	3
d) Chemical Structure	4
e) Characterization	5
<b>B. Manufacturing Process</b>	<b>5</b>
1. Raw Materials	5
2. Process	6
3. Specifications	6
4. Microbiological Purity	8
5. Pesticidal Purity	8
6. Product Analyses	9
7. Stability	10
<b>II. Use of PDX</b>	<b>11</b>
<b>A. Historical Exposure to PDX</b>	<b>11</b>
<b>B. Intended Use of PDX</b>	<b>11</b>
1. Addition Level of PDX to Infant Formula	11
2. Estimated Daily Intake of PDX	12
<b>III. Safety Data</b>	<b>13</b>
<b>A. Effects of PDX on the Gastrointestinal Tract</b>	<b>13</b>
1. Effects on Stool Biota	13
2. Effects on Laxation	13
3. Effects on Mineral Absorption	13
4. Physiological Characteristics of Infants	14
<b>B. Absorption, Distribution, Metabolism and Excretion (ADME)</b>	<b>15</b>
1. Experimental Animals	15
2. Humans	18
3. Summary of ADME Studies	21
<b>C. Preclinical Studies</b>	<b>22</b>
1. Biological Effects	22
a. General Gastrointestinal Physiology	23
b. Gastrointestinal Microbiota	32
c. Nutrient Absorption	33
(1) Transport Processes	33
(2) Glucose	33
(3) Calcium	33
(4) Lipid	34
d. Laxation	35
e. Summary of Biological Effects of Polydextrose in Rats	38
2. Toxicity Studies	38
a. Genotoxicity	48
(1) <i>In Vitro</i>	48

(2) <i>In Vivo</i> _____	48
(3) Summary of Genotoxicity _____	49
b. Acute Toxicity _____	49
(1) Mice _____	50
(2) Rats _____	50
(3) Dogs _____	51
(4) Summary of Acute Toxicity _____	51
c. Subchronic Toxicity _____	51
(1) Polydextrose (acidic form) _____	52
(a) Rat _____	52
(b) Dog _____	52
(2) Polydextrose (neutral form) _____	53
(a) Monkey _____	53
(b) Dog _____	54
(3) Summary of Subchronic Toxicity _____	55
d. Chronic Toxicity _____	56
(1) Polydextrose (acidic form) _____	56
(2) Polydextrose (neutral form) _____	58
(3) Summary of Chronic Toxicity _____	61
e. Carcinogenicity _____	61
(1) Mouse _____	61
(2) Rat _____	62
(3) Summary of Carcinogenic Effects _____	62
f. Reproductive and Developmental Toxicity _____	63
(1) Dominant Lethal Assay _____	63
(2) Segment I (Reproductive) Studies _____	63
(3) Segment II (Developmental) Studies _____	65
(4) Segment III (Perinatal and Postnatal) Study _____	69
(5) Multi-Generation Study _____	71
(6) Summary of Reproductive and Developmental Toxicity _____	75
g. Summary of Preclinical Safety Studies _____	75
(1) Mechanism of Calcium Nephropathy _____	76
(2) Differential Effect of Polydextrose (Acidic and Neutral Forms) on Diarrhea _____	76
<b>D. Clinical Studies</b> _____	<b>77</b>
<b>1. General Safety</b> _____	<b>77</b>
<b>2. Serum Lipids</b> _____	<b>78</b>
<b>3. Microbiota in Adult Humans</b> _____	<b>78</b>
<b>4. Mineral and Glucose Absorption</b> _____	<b>79</b>
<b>5. Laxation</b> _____	<b>80</b>
a. Adults _____	83
b. Children _____	88
<b>6. Summary of Clinical Safety Studies</b> _____	<b>89</b>
<b>IV. Safety Assessment/GRAS Determination</b> _____	<b>91</b>
<b>A. Safety of PDX</b> _____	<b>91</b>
<b>B. General Recognition of the Safety of PDX</b> _____	<b>92</b>
<b>V. References</b> _____	<b>94</b>

*List of Appendices*

*Appendix A. Conclusion of the Expert Panel*

## I. Description of Substance

### A. Identity

#### 1. Common or Usual Name

The substance that is the subject of this GRAS determination is polydextrose (PDX), a randomly bonded polymer of glucose and sorbitol with traces of citric or phosphoric acid catalyst attached to the polymer by mono- and diester bonds. The specific PDX product addressed is manufactured by Danisco Sweeteners (Terre Haute IN) and sold under the trade name Litesse® Two.

#### 2. Chemical Names

An alternative chemical name for the substance, in addition to polydextrose or PDX, is D-glucose polymer reaction product with citric acid or phosphoric acid and sorbitol.

#### 3. CAS Registry Number

The Chemical Abstracts Service registry number for PDX is 68424-04-4. PDX is also designated in the International Numbering System (FAO/WHO) by number INS1200.

#### 4. Composition

PDX is a polymer of glucose with sorbitol and traces of citric acid catalyst attached to the polymer by mono- and diester bonds. The glucose molecules are randomly bonded, although the  $\beta(1-6)$  bond predominates (Allingham 1982). Because of the random glucose-glucose and glucose-sorbitol bonds, PDX is more resistant to enzyme or acid hydrolysis than other glucose polymers such as in soluble starch.

##### a) Molecular Formula

Since PDX is a randomly bonded condensation polymer of D-glucose with some bound sorbitol and citric or phosphoric acid; there is no specific chemical formula for the product. Polydextrose is a polymer of glucose, which has a molecular formula  $C_6H_{12}O_6$  and molecular weight of 180. However, Polydextrose is a condensation polymer, which means that whenever a glucose molecule attaches to the polymer chain, it loses a molecule of water. Therefore, the repeating unit in the polymer is not  $C_6H_{12}O_6$  but  $C_6H_{10}O_5$  with MW 162. The polymerization takes place in vacuo and the water released by polymerization is removed by condensation.

##### b) Molecular Weight

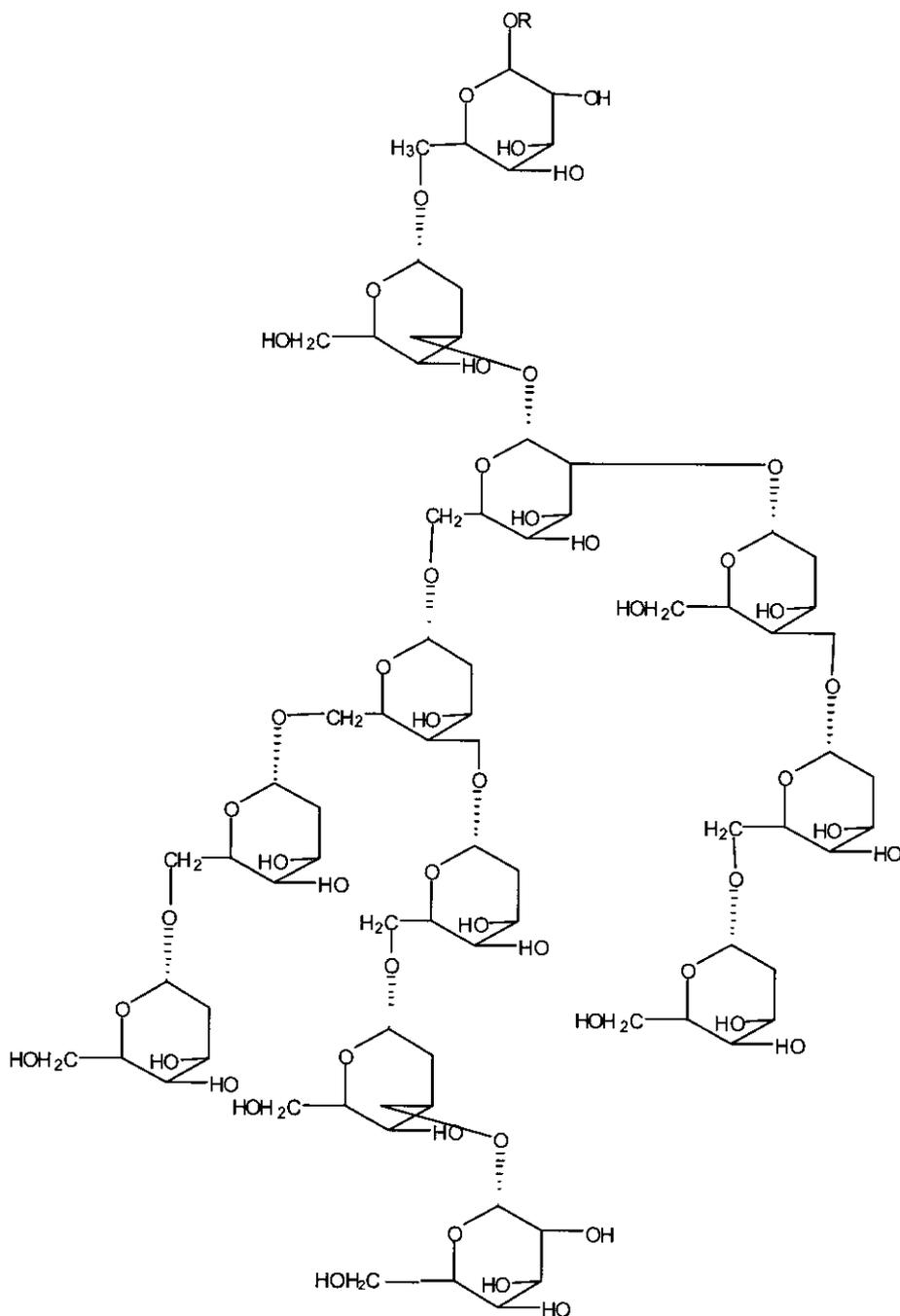
The average molecular weight of PDX is 2,000 g/mole, but because of the random bonding of glucose molecules PDX exists in a wide range of molecular weights. Figdor and Rennhard (1981) reported that the molecular weight for PDX ranges between 250 and 18,000 g/mole although it is most often between 1,200 and 2,000 g/mole (Kibbe 2000)

##### c) Degree of Polymerization

Polymers may be characterized by their degree of polymerization (DP). Ninety percent of PDX polymers have a DP of  $\leq 30$ ; while 30% have a DP  $\leq 4$  (Figdor and Rennhard 1981; Radosta et al. 1992). The average DP is reported to be  $\sim 12$  (Craig 2001).

### d) Chemical Structure

A representative chemical structure of PDX (adapted from Mitchell et al. 1996) is shown in Figure 1.



**Figure 1. Representative structure of PDX. The R Group = H, sorbitol, sorbitol bridge or more PDX.**

### e) Characterization

PDX is an odorless, white to light cream amorphous powder with a non-sweet bland taste and a melting point higher than 130°C (Budavari et al. 1999). It is very soluble in water; aqueous solutions with over 70% Polydextrose (ie, 70g of Polydextrose dissolved in 30 g of water) are easily achievable. PDX provides about 1 kcal/g energy or less (Figdor and Rennhard 1981; Figdor and Bianchine 1983; Jühr and Franke 1992; Ranhotra et al 1993; Achour et al 1994).

## B. Manufacturing Process

PDX was first synthesized in the late 1960s, the end product of a research and development effort to produce a low-calorie bulking substance for use in food (Rennhard 1973; Anonymous 1981; Mitchell 1996). The quality of the PDX is dependent on the manufacturing method, which has been improved over the years. The first PDX product made by Pfizer was called Polydextrose Type A as it was slightly acidic because of the presence of trace amounts of citric acid. Upon customer request, a neutral form of polydextrose was also produced by adding small amounts of potassium hydroxide or potassium carbonate. Polydextrose (neutral form) was called Type N and contained up to 1.5% potassium. Improvements in the manufacturing process of PDX reduced citric acid in the final product and eliminated the need for potassium hydroxide neutralization (designated in this notification as “polydextrose (acidic form))). Subsequent refinements of the manufacturing process has led to the Litesse® Two product. Pfizer sold manufacturing rights for PDX to Cultor Food Science in 1996. Cultor, and the rights to produce PDX, were sold to Danisco in 1999.

PDX was first synthesized by vacuum-melt condensation of glucose in the presence of sorbitol and catalytic amounts of citric acid, resulting in a randomly linked polymer (Rennhard 1973; Allingham 1982; Burdock and Flamm 1999). The synthesized product typically contained PDX polymer (90% minimum), glucose (4% maximum), sorbitol (2% maximum), levoglucosan (4% maximum), water (4% maximum) and citric acid (0.1%) (Allingham 1982). Trace amounts of 5-hydroxymethyl-furfural from glucose caramelization were produced as byproducts of the polymerization process (Bill 1987; Thomas et al. 1991; Setser and Racette 1992). As noted above, there were two forms of PDX, acidic and neutral. Polydextrose (neutral form) was the potassium salt (up to 1.5% KOH) and was designated as polydextrose-N or PDX-N, while polydextrose (acidic form) did not contain potassium hydroxide and was generically referred to as polydextrose or PDX. Polydextrose (neutral form) is no longer produced and marketed in the United States. Danisco Sweeteners in the United States manufactures the descendent of polydextrose (acidic form) under the trade name Litesse® Two (Danisco Sweeteners 2004g). Since the neutral form of PDX is no longer manufactured in the United States, use of the term “polydextrose” or “PDX” without further qualification in this document refers to the acidic form and its descendents.

The PDX proposed for use in infant formula is manufactured by Danisco Sweeteners of Terre Haute, IN, and sold as Litesse® Two.

### 1. Raw Materials

The starting materials in the manufacture of PDX are glucose (powdered, crystalline and/or liquid) or glucose-containing materials such as hydrolyzed starch, a polyol such as sorbitol and a catalytic acid such as citric acid or phosphoric acid.

## 2. Process

Food grade polydextrose is manufactured in accordance with current good manufacturing practice (cGMP). The first step of the manufacturing process (see Figure 2) is the vacuum-melt condensation method (Danisco Sweeteners 2004e). In this process, glucose (powdered, crystalline and/or liquid) or glucose-containing materials such as hydrolyzed starch is heated under vacuum at 150 to 160°C for about 20 minutes in the presence of a polyol such as sorbitol and with low levels of a catalytic acid such as citric or phosphoric acid. Because of the low levels of catalyst used in preferred embodiments, minimal or no off-flavors and little color are formed during the course of the reaction. The product may be purified using ion exchange, membrane filtration, or carbon treatment. Litesse® Two and Litesse® Ultra are prepared by additional resin purification and hydrogenation respectively (see Figure 2). Danisco Sweeteners provides this polydextrose product in powdered form or as a 70% aqueous solution.

Manufacturing process of acidic polydextrose as Litesse® Two (Danisco Sweeteners 2004e).

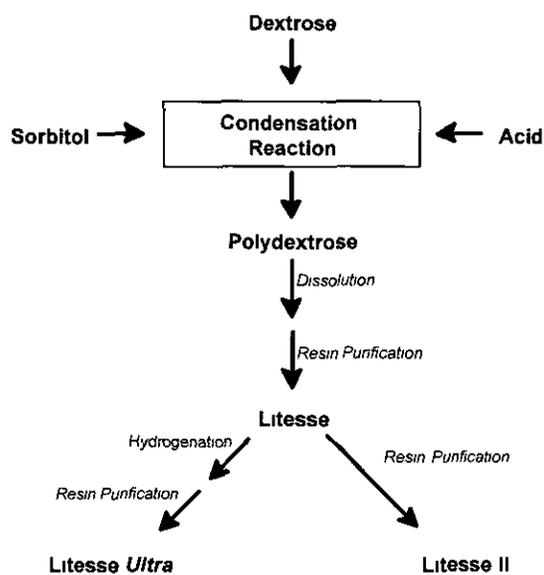


Figure 2. Schematic of PDX Product Manufacture

## 3. Specifications

Specifications for PDX from Danisco Sweeteners, the Joint FAO/WHO Expert Committee on Food Additives (JECFA 1998), and the Food Chemicals Codex (FCC 2003) are presented in Table 1. Up to 10% of PDX may consist of small monomeric substances, including 1,6-anhydro-D-glucose, glucose, and sorbitol. Because of its hygroscopic nature, FCC (2003) specifications require that PDX be stored in tight, lightproof containers.

Table 1. Specifications for PDX

Parameter	Source of Specification		
	Danisco Sweeteners (2004f)	JECFA (1998)	FCC (2003)
Name	Litesse <sup>®</sup> Two	Polydextroses	Polydextrose
Identification	Passes FCC test	See footnote 1	See footnote 2
Assay (calculated on the anhydrous ash-free basis)	NLT <sup>3</sup> 90.0% polydextrose	NLT 90.0% polymer	NLT 90.0% polymer
5-Hydroxymethylfurfural and related compounds	NMT <sup>4</sup> 0.1%	NMT 0.1%	NMT 0.1%
1,6-Anhydro-D-glucose	NMT 4.0%	NMT 4.0%	NMT 4.0%
Glucose and Sorbitol	NMT 0.25% glucose NMT 6.0% sorbitol	NMT 6.0% combined	NMT 6.0% combined
Molecular weight	Passes FCC test	No polymer greater than 22,000 Daltons	Passes test
pH	4.5-6.5	2.5-7.0 (10% solution)	2.5-7.0 (10% solution)
Acidity	NMT 0.002 meq/g	No specification	No specification
Water	NMT 4.0%	NMT 4.0	NMT 4.0%
Heavy Metals	NMT 0.5 ppm	No specification	No specification
Lead	NMT 0.5 ppm	NMT 0.5 ppm	NMT 0.5 ppm
Nickel	NMT 2 ppm	No specification	No specification
Solubility	Very soluble in water, sparingly soluble to insoluble in most organic solvents	Very soluble in water	Very soluble in water
Residue on ignition	NMT 0.3%	No specification	NMT 0.3%
Packaging and storage	Store in tight containers in a dry place below 40°C	No specification	Store in tight, light-proof containers
<p>1 Identification test JECFA (A) Solubility (see description in table) (B) Test for sugar: To 1 drop of 1 in 10 solution of the sample, add 4 drops of 5% phenol solution, then rapidly add 15 drops of sulfuric acid TS. A deep yellow to orange color is produced. (C) Solubility in acetone – With vigorous swirling add 1 ml of acetone to 1 ml of a 1 in 10 solution of the sample. The solution remains clear. With vigorous swirling add 2 ml of acetone to the solution. A heavy, milky turbidity develops immediately. (D) Test for reducing sugar – To 1 ml of a 1 in 50 solution of the sample, add 4 ml of alkaline cupric citrate TS and boil vigorously 2-4 minutes. Remove from heat and let precipitate (if any) settle. The supernatant is blue or blue-green (JECFA 1998)</p> <p>2 Identification test FCC (A) Add 4 drops of 5% aqueous solution to 1 drop of a 1:10 aqueous solution, then rapidly add 15 drops of sulfuric acid. A deep yellow to orange color appears. (B) While vigorously swirling (vortex mixer), add 1.0 ml of acetone to 1.0 ml of a 1:10 aqueous solution. The solution remains clear. Retain this solution for <i>Identification Test C</i>. (C) While vigorously swirling, add 2.0 ml of acetone to the solution from <i>Identification Test B</i>. A heavy, milky turbidity develops immediately. (D) Add 4 ml of alkaline cupric citrate TS to 1 ml of a 1:50 aqueous solution. Boil vigorously for 2 to 4 minutes. Remove from heat, and allow the precipitate (if any) to settle. The supernatant liquid is blue or blue-green (FCC 2003)</p> <p>3 NLT = not less than</p> <p>4 NMT = not more than</p>			

In addition to the specifications above, Danisco Sweeteners (2004e) states that the levels of other metals and the maximum limit in the Litesse<sup>®</sup> Two product they produce is: arsenic (1 ppm), mercury (0.1 ppm) and cadmium (0.1 ppm).

#### 4. Microbiological Purity

During the final drying step, Danisco Sweeteners' (2004c) PDX product Litesse® Two is subjected to heat treatment that destroys most microbiological contamination. In this process the polydextrose solution is heated to 170°C for one minute under vacuum to raise solid levels from 60% to 95%. Danisco Sweeteners states that this product meets the microbiological limits presented in Table 2.

Table 2. Microbiological Limits of Danisco Sweeteners PDX Products

Microbe	Limit	Medium	Incubation Conditions
Total Aerobic Plate Count	100/g Maximum	TGY* Agar	35°C for 48 hours
Yeast and Mold	20/g Maximum	Antibiotic PDA*	22-25°C for 120 hours
<i>Escherichia coli</i>	Negative to Test/25 g	Fluid Lactose Medium	30-35°C for 24-48 hours
		EMB*	35°C for 24 hours
		IMVIC*	35°C for 24 hours
Coagulase positive <i>Staphylococcus</i>	Negative to Test/25 g	Trypticase Soy Broth	35°C for 48 hours
		Baird-Parker Plates	35°C for 48 hours
		BHI*	35°C for 18 hours
		Coagulase Plasma	35°C for 6 hours
<i>Salmonella</i>	Negative to Test/25 g	Lactose	35-37°C for 18 hours
		Tetrathionate Broth	42°C for 6-8 hours
		Selenite Cystine Broth	35-37°C for 6-8 hours
		M* Broth	42°C for 18 hours
		XLD*, HE* Plates	35°C for 24 hours
		Bismuth Sulfite Agar Plates	35°C for 48 hours
*TGY=tryptone glucose yeast PDA=potato dextrose agar EMB=eosin-methylene blue IMVIC=indole methyl red Vogues Proskauer citrate		BHI=brain heart infusion M=D-mannose XLD=xylose lysine deoxycholate HE=Hektoen enteric	

#### 5. Pesticidal Purity

Danisco Sweeteners has also tested the Litesse® Two PDX product for pesticide residues. The analytical results of all 132 pesticides tested were below the limits of detection (Table 3). These results are in agreement with the published findings of Gelardi and Mountford (1993).

Table 3. Pesticide Residues Below Detectable Limits in PDX

Acephate	Ametryn	Atrazine	Azinphos-methyl
Benthiocarb	Bolstar (Sulprofos)	Carbofenthion (Trnthon)	Chlorfenvinphos (Supona)
Chloroprotham (CIPC)	Chlorpyrifos (Dursban)	Chlorpyrifos-methyl	Clodrin (Crotophos)
Coumaphos (Co-Ral)	Cyanazine (Bladex)	DEF	Demeton (Systox)
Diazinon	Dibrom (Naled)	Dicrotophos (Didrin)	Dimethoate (Cygon)
Dioxathion (Delnav)	Diphenyl Amine	Disulfoton (Disyston)	EPN
Ethion	Ethoprop (Modap)	Fenamiphos (Nemacur)	Fenitrothion (Sumithion)
Fenthion (Baytex)	Fonofos (Dyfonate)	Hexazinone (Velpar)	Hostathion (Triaziphos)
Imazalil	Imidan (Phosmet)	Isofenphos (Ottanol)	Malathion
Metalaxyl (Ridomyl)	Methamidophos (Monitor)	Methidathion (Supracide)	Methyl Parathion
Metolachlor (Dual)	Metribuzin (Sencor)	Mevinphos (Phosdrin)	Molinate (Ordram)
Myclobutanil	Parathion	Phorate (Thimet)	Phosalone (Zolone)
Phosphamidon (Dimecron)	Pniphos-methyl	Profenofos (Curacron)	Prometryne
Propetamiphos (Safrotin)	Ronnel (Fenchlorfos)	Simazine	Terbacil
Tetrachlorvinphos	Thiabendazole	Thionazin (Zinophos)	
A, b, d-BHC	Alachlor	Alert (Pirate)	Aldrin
Benfluralin	BHC	Bifenox	Bifenthrin
Bromacil	Captafol	Captan	Chlordane
Chlorobenzilate	Chlorothalonil	Cyanazine	Cyfluthrin
Cypermethrin	Dachthal	DDD	DDE
DDT	Dichlorbenil	Dichlone	Dichloran
Dicofol	Dieldrin	Dyrene	Endosulfan alpha
Endosulfan beta	Endosulfan sulfate	Endosulfants (Total)	Endrin
Esfenvalerate	Ethafuralin	Fluvalinate	Folpet
Heptachlor	Heptachlor epoxide	Iprodione	Lindane
Linuron	Methoxychlor	Metribuzin	Mirex
Myclobutanil	Nitrofen	Oxadiazon	Oxyfluorfen
Pendamethalin	Pentachloronitrobenzene	Permethrin	Perthane
Polychlorinated biphenyls	Profuralin	Pronamide	Pyrethrins
Tetradifon	Toxafene	Tridimephon	Trifluralin
Vegadex	Vinclozolin		
Aldicarb Sulfone	Aldicarb Sulfoxide	Aldicarb	Carbaryl
Carbofuran	3-OH Carbofuran	Methiocarb	Methomyl
o-Phenyl Phenol	Oxamyl	Propoxur	

## 6. Product Analyses

Analytical results of several samples from non-consecutive batches indicate that the PDX product Litesse<sup>®</sup> Two meets all food-grade specifications as shown in Table 4.

Table 4. Batch Analyses of Litesse® Two

Parameter	Lot					
	V47270I	V48010I	V48270I	V49040I	V49220I	V49090I
Assay (%)	92.3	92.0	92.9	92.1	93.7	93.0
5-hydroxymethylfurfural (%)	0.04	0.04	0.04	0.04	0.04	0.04
Water (%)	0.9	1.1	1.3	0.8	1.2	1.0
Residue on ignition (%)	0.00	0.04	0.01	0.00	0.00	0.00
Yeast and Mold	Passes	Passes	Passes	Passes	Passes	Passes
pH (10% solution)	4.3	4.3	4.2	4.6	4.3	4.3
Molecular weight limit	Passes	Passes	Passes	Passes	Passes	Passes
1,6-anhydro-D-glucose (%)	2.6	2.8	2.5	2.3	2.5	2.6
Glucose + sorbitol (%)	4.3	4.6	5.1	4.5	4.8	4.3
Lead (ppm)	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Heavy metals (ppm)	<5	<5	<5	<5	<5	<5
Anaerobic plate count (CFU/g)	0	0	6	0	0	0
<i>E. coli</i> (negative in 25 g)	Passes	Passes	Passes	Passes	Passes	Passes
<i>Salmonella</i> spp (negative in 25 g)	Passes	Passes	Passes	Passes	Passes	Passes
<i>S. aureus</i> (negative in 25 g)	Passes	Passes	Passes	Passes	Passes	Passes

## 7. Stability

The stability of Danisco Sweeteners' PDX products is dependent on whether they are in powder form or in 70% solution, as well as the temperature at which they are stored (Danisco Sweeteners 2004a; Danisco Sweeteners 2004b; Danisco Sweeteners 2004d). The powdered form of polydextrose has a shelf life of up to 36 months. Danisco Sweeteners polydextrose products are also stable when subjected to an acidic environment and high temperature (Table 5); only 2% of polydextrose is degraded into free monomers when subjected to an acid environment and high heat.

Table 5. Acid and Heat Stability of the Danisco Sweetener PDX Product

Polydextrose Product	Increase in Free Monomers (%) After Incubation at pH 2.6 and 100°C		
	0 Hour	1 Hour	5 Hours
Litesse® Two	0.02	0.02	2.28

## II. Use of PDX

### A. Historical Exposure to PDX

PDX does not occur naturally, and so its only intake is that resulting from its use as an ingredient added to foods or an excipient in dietary supplements or pharmaceutical tablets. PDX is approved as a food additive for direct addition to human food as a bulking agent, texturizer, formulation aid or humectant (21 CFR §172.841). Foods to which polydextrose may be added include baked goods and baking mixes (restricted to fruit, custard and pudding-filled pies, cakes, cookies and similar baked products), chewing gum, confections and frostings, dressings for salads, frozen dairy desserts and mixes, fruit spreads, gelatins, puddings and fillings, hard and soft candy, peanut spread, sweet sauces, toppings and syrups (Burdock 1997). PDX is also included in the FDA Inactive Ingredients Guide for use as an excipient in currently marketed drug products that are in the form of tablets or film-coated tablets (FDA 1996b).

In 1990, PDX was approved for use in food within the European Union (Vincent 1991) as a Generally Permitted Food Additive. According to Vincent (1991), specific European countries that have approved the use of polydextrose for use in food include Belgium (reduced energy foodstuffs), France (dietetic products), United Kingdom, Sweden (15% in ice cream) and Switzerland (bulking substance). PDX is approved for use in food in Argentina, Brazil, China, Egypt, Japan, Korea, Poland and Taiwan (Craig et al. 1998; Craig et al. 1999). In Japan, PDX is approved for use under the Japanese Foods for Specified Health Use (FOSHU) law. Under this law, the label claim of "provides improved intestinal function" may be made for food products that contain PDX.

In 1981, JECFA evaluated the use of PDX in food and established an acceptable daily intake (ADI) of 70 mg/kg bw/day (JECFA 2004a; JECFA 2004b). In 1987, JECFA re-evaluated polydextrose and revised the ADI to "Not Specified" (JECFA 1987). An ADI of Not Specified is used for food ingredients that have been determined to be of very low toxicity, and which the total dietary intake resulting from its use at the levels necessary to achieve the desired effect, as well as from its acceptable background in food, does not represent a health hazard to humans (JECFA 2001).

### B. Intended Use of PDX

#### 1. Addition Level of PDX to Infant Formula

MJN intends to add PDX to milk-based infant formula (along with GOS) as a prebiotic ingredient. When defining the maximum addition levels, the manufacturer specifications were taken into consideration. In addition, according to experimental trials in our infant formula production environment, we have observed loss of the notified substances during processing of the infant formulas containing these ingredients. We have considered both factors when defining the maximum addition levels.

The maximum intended addition level of Litesse® Two is 2.5 g/L. Considering losses during the manufacturing of our infant formula and that Litesse® Two comprises approximately 86% PDX, the maximum intended addition level of Litesse® Two is equivalent to 2 g/L of PDX.

000261

## 2. Estimated Daily Intake of PDX

According to tables of daily energy intake by formula-fed infants provided by Fomon (1993), the subpopulation of infants with the highest intake per kg body weight is boys age 14–27 days. The 90<sup>th</sup> percentile energy intake by this group is 141.3 kcal/kg/day. Among girls, the highest energy intake is found in the same age group, 14–27 days, and is nearly as high as boys: 138.9 kcal/kg/day. MJN's Enfamil LIPIL with Iron, along with most other standard formulas, contains 0.676 kcal/mL when ready to consume. Therefore, to obtain 141.3 kcal energy/kg, an infant boy must consume 209.0 mL/kg of formula. To reach her 90<sup>th</sup> percentile of energy consumption, 138.9 kcal/kg/day, an infant girl must consume 205.5 mL/kg of formula. The 90<sup>th</sup> percentile of formula intake for the two sexes combined is about 207 mL/kg/day.

The 90<sup>th</sup> percentile daily intake of Litesse® Two added at 2.5 g/L is estimated to be 0.5 g/kg bw and that of PDX, constituting 86% of Litesse® Two, is estimated to be 0.4 g/kg bw.

As the infant grows, formula intake increases, but more slowly than weight gain, so that consumption assessed as mL formula per kg body weight is lower for infants older than 27 days. As a result, intake per kg body weight of PDX decreases as the infant grows older and larger. Between weeks 8-12, the estimated 90<sup>th</sup> percentile daily intake of PDX is estimated to be 0.35 g/kg bw. Establishing the EDI of PDX at 0.4 g/kg bw/day is thus a conservative basis for estimating long-term exposure.

Since there is no other source of PDX in the diet of formula-fed infants, the above estimated daily intake constitutes the total daily exposure of infants to PDX at the 90<sup>th</sup> percentile.

### III. Safety Data

#### A. Effects of PDX on the Gastrointestinal Tract

Polydextrose, like the oligosaccharides in human milk, is resistant to enzymatic hydrolysis in the upper gastrointestinal tract and thus reaches the colon largely intact. Like human oligosaccharides, available experimental evidence indicates that infants given formula supplemented with polydextrose may tend to develop a colonic microbiota population that more closely resembles that found in breast-fed infants than those receiving formula without added prebiotic ingredients. Additionally, infant formula containing polydextrose may produce more frequent and softer stools than does control formula. The results of a number of studies suggest that polydextrose ingested by infants may thus produce beneficial changes in colonic microbiota and improve laxation as well as increase mineral absorption.

##### 1. Effects on Stool Biota

In a clinical study (not randomized or controlled), no increase in *Bifidobacterium* or *Lactobacillus* with polydextrose ingestion was observed, but a reduction in bacteroides was noted (Endo et al. 1991). The findings of *in vitro* experiments designed to simulate the intestinal tract of the human infant support the effects of polydextrose on stool biota. In a continuous culture system with adult fecal samples designed to simulate the colonic environment, Probert et al. (2004) reported that polydextrose at concentrations of 1 and 2% had consistent stimulatory effects on colonic bifidobacteria, including *B. infantis*. In this study, in experimental vessels simulated to represent the ileum and proximal colon, polydextrose supported a wide variety of bifidobacteria and led to an increase in the level of lactobacilli. In another *in vitro* model of the infant gut developed to test prebiotics for infant-formula applications, polydextrose was effective in increasing both bifidobacteria and lactobacilli (Gemmell et al. 2004). While exhibiting a slower rate of fermentation, polydextrose provided prebiotic effects equal to or surpassing those of other carbohydrates tested.

##### 2. Effects on Laxation

In two human clinical studies of polydextrose with administration of 15 to 30 g/day, the results consistently showed that intake of polydextrose by adult humans results in softer stools (Tomlin and Read 1988; Saku et al. 1991). In these clinical studies, the effects of polydextrose on laxation appeared to increase with increasing dose. The doses of polydextrose (15 to 30 g/day) produced the most significant effects on laxation, but were also accompanied by increases in diarrhea and flatulence.

##### 3. Effects on Mineral Absorption

It has been hypothesized that supplementation of the diet with prebiotics may increase the absorption of minerals such as calcium, magnesium, phosphorus and potassium (Demigne et al. 1989; Heijnen et al. 1993; Beynen et al. 2001). The available studies suggest that prebiotics may be particularly beneficial under conditions of increased calcium requirements. In an animal study, Hara et al. (2000) assessed the effects of polydextrose (5% in the diet for 21 days) on calcium absorption. The results of this study support the use of polydextrose in increasing calcium absorption and its incorporation into bone.

#### 4. Physiological Characteristics of Infants

Infants have unique physiological characteristics that distinguish them from adults. Several of the physiological differences between infants and adults have been recognized to be important when conducting safety assessments for infants. The IOM Committee on the Evaluation of the Addition of Ingredients New to Infant Formula described four unique characteristics of infancy that are critical to such a safety evaluation (IOM 2003). These unique characteristics include (1) infant vulnerability, (2) gastrointestinal and renal function, (3) immune function and (4) brain development and behavior. These four characteristics are described in the following sections.

Infancy is a time when rapid development of many anatomical tissues and systems occurs. Because infants have limited ability to communicate verbally, there is potential that a harmful effect due to some chemical exposure might be overlooked. During delivery, the environment dramatically changes from one in which oxygen and nutrients are delivered directly from the mother to the fetus via the placental blood supply to an infantile environment that requires efficient respiratory and gastrointestinal systems, as well as coordination of numerous biological functions. Not all organ systems are fully mature at birth, and those that are not may be highly susceptible to environmental toxicants. Normal development is dependent on the timely emergence of critical structures and developmental processes during the period of rapid growth in early infancy. It is during this period of rapid development that the vulnerability of the infant to nutritional imbalances, illnesses, inadequate care practices and other environmental disturbances is at its highest. The resulting disturbances may be irreversible, or the ability to compensate for them may be limited. The food consumption rate in infants is greater than that in adults on a/kg bw basis. Furthermore, infant formula is the only food source for many infants less than 6 months of age (IOM 2003).

Gastrointestinal (GI) tract morphological differences among infants, children and adults were investigated in the mid-1960s (Walker-Smith and Harrison 1966; Walker-Smith 1967a; Walker-Smith 1967b; Trier 1968; Walker-Smith 1969). Walker-Smith (1972) reported a change in the morphology of the duodenum and jejunum from finger-shaped villi in neonates to broader shaped (i.e., thin ridges and tongues) villi in infants and very young children, followed by a return to finger-shaped villi by four years of age. Walker-Smith (1972) attributed these morphological changes of the upper GI tract to bacterial colonization (mainly Gram-positive microorganisms). These age-dependent changes in the morphology of the GI mucosa were less pronounced in the ileum, and absent in the cecum (i.e., the villi remained finger-shaped). Stenling et al. (1984) also demonstrated age-dependent differences in the mucosal morphology of the upper intestine of children as compared that in adults. The IOM (2003) considered these anatomical differences to be minor and reported the infant GI tract to be fully developed. This is especially true for the lower GI tract because it is morphologically equivalent to adults at birth. The IOM also reported that dietary constituents do not influence the development of the GI tract and the kidneys. Renal tubular re-absorption and urine acidification reach normal values at several months of age, whereas glomerular filtration reaches adult values at around 3 years of age.

Gastrointestinal glycosidases develop during the prenatal period (Auricchio et al. 1965). At birth, gastrointestinal enzymes are fully functional with the exception of amylase and maltase, the activity of which are normally lower at birth than in adults (Auricchio et al. 1965; Mobassaleh et al. 1985). The gastrointestinal tract of newborn infants is sterile (i.e., lacking any

microbial population) (Gracey 1982) other than microbes obtained from the mother's birth canal during birthing. Colonization of the gastrointestinal tract occurs over the first year of life (Levanova et al. 2001).

At birth, the immune system of the infant is not fully developed (Crockett 1995). The IOM (2003) reported that the immune system of the neonate has reduced ability to prevent pathogen invasion, as well as a reduced response to antigenic stimulation. The report also describes increased permeability of the gastrointestinal mucosal barrier during inflammation or infection that results in the disruption of the epithelial cell layer. The increased permeability allows the absorption of macromolecules and stimulates an allergic response to food proteins.

The brain is not fully developed at birth, but rapid development takes place during the first year of life (Nelson 1995; Nelson 2001; Johnson 2001). During this early period, subcortical and cortical central nervous system (CNS) structures mature, resulting in the functional development of visually-guided reaching, face recognition and orientation toward faces, explicit and working memory, focused attention and inhibitory control. Nutrition during the first year of life of the infant can influence CNS development and function (Wauben and Wainwright 1999; Rao and Georgieff 2000). Behavior and social-emotional function also develop during the first year of infancy (Ruff and Rothbart 1996; Gunnar 2000). The IOM (2003) reported that, for some infants, a large proportion of nutrition is from formula; thus, formula alterations that deter normal development of the central nervous system and behavioral patterns can potentially have long-term consequences.

## **B. Absorption, Distribution, Metabolism and Excretion (ADME)**

Investigations of the absorption, distribution, metabolism and excretion of polydextrose in experimental rats and humans are described. This information is useful in interpreting biological effects described starting on page 22 and D. Clinical Studies (page 78). Based on the results from these ADME studies, the fate of polydextrose within the body is illustrated in Figure 3.

### **1. Experimental Animals**

Figdor and Rennhard (1981) investigated the absorption of polydextrose in the rat (strain not specified; weight 125-200 g). A single dose of [ $^{14}\text{C}$ ]-polydextrose was administered to male rats ( $n=2-4$  per group) by gavage (55 mg/ kg bw) or intravenously (25 or 50 mg/kg bw). Radioactive carbon dioxide ( $^{14}\text{CO}_2$ ) was collected at hourly intervals for 13 hours. In rats injected intravenously with polydextrose, a single collection period between 13 and 24 hours after treatment was also included. Collection of  $^{14}\text{CO}_2$  was performed using an Aerospace Industries rat restrainer and metabolism cage. The  $^{14}\text{CO}_2$  data was used to calculate the caloric utilization of polydextrose, the amount of polydextrose biotransformed by mammalian and microbiota enzymes to carbon dioxide ( $\text{CO}_2$ ). Urine and feces were collected for a total collection time of 72 hours.

When administered intravenously, 90% of the polydextrose was excreted in urine, and a minor amount was excreted in feces (Table 6). Two percent was converted to  $\text{CO}_2$ . Figdor and Rennhard reported that the high elimination in urine was not surprising because 99% of the administered polydextrose had a molecular weight of less than 15,000. Molecules less than 20000 are rapidly eliminated by glomerular filtration from the kidneys (Arturson and Wallenius 1964; Arturson et al. 1971). Nearly all of the polydextrose excreted in the urine was recovered

within three hours of administration. Based on the rapid elimination in urine, the investigators concluded that the elimination half-life was less than 30 minutes and the relative volume of distribution was less than total body water (i.e., polydextrose appeared to be primarily confined to the extracellular space). The investigators reported that the route of administration (i.e., intravenous) and the small extent to which polydextrose was converted to CO<sub>2</sub> indicates that degradation of polydextrose by mammalian enzymes *in vivo* is "slow or non-existent" (Figdor and Rennhard 1981).

Figdor and Rennhard (1981) also found that, when administered orally, 60% of the polydextrose was excreted from the body in the feces, while 35% was biotransformed into CO<sub>2</sub> (Table 6). Only 2% was absorbed and excreted from the body in urine. The majority of the radioactivity excreted in the urine was in urea. The investigators reported that the amount of polydextrose absorbed across the gastrointestinal tract and excreted in the urine as parent compound (i.e., polydextrose) was at most 0.24% of the administered dose, indicating that polydextrose is virtually unabsorbed across the mucosal barrier. This conclusion is also based on the finding that very little, if any, polydextrose is metabolized endogenously, as demonstrated after intravenous administration of polydextrose. Of the 60% of orally administered polydextrose that was unabsorbed and excreted in the feces, 13% was converted by microbiota into volatile fatty acids (acetic acid, n-butyric acid and propionic acid), leaving 47% as unchanged polydextrose in the feces. Maximal urinary and fecal excretion of polydextrose occurred between six and eight hours after administration, indicating that absorption of polydextrose degradation products (i.e., CO<sub>2</sub> and smaller fatty acids) occurred in the lower rather than upper GI tract (Figdor and Rennhard 1981). Cooley and Livesey (1987) obtained similar results in male Wistar rats, indicating that these findings are reproducible across different laboratories.

Table 6. Excretion of [<sup>14</sup>C]PDX Administered Acutely to Male Rats

Collection Interval	Intravenous (units are % of administered dose)				Oral (units are % of administered dose)			
	0-24	24-48	48-72	Total	0-24	24-48	48-72	Total
Caloric Utilization*	1.93±0.3 <sup>†</sup>	NM	NM	1.93±0.3	34.8±5.2 <sup>‡</sup>	NM	NM	34.8±5.2
Urine	90.3±12	0.3±NR	0.2±NR	90.8±12	1.5±0.2	0.2±0.1	0.1±0.06	1.8±0.3
Feces	3.0±4.3	1.1±1	0.3±0.1	4.4±5.4	50.2±12	9.0±8.8	1.1±0.9	60.3±8.6
Total	NR	NR	NR	97.13±9.7	NR	NR	NR	96.9±6.2

\*Caloric utilization for polydextrose is calculated from the quantity of CO<sub>2</sub> obtained from rats after labeled polydextrose administration. These determinations are based on a total of 60% of an available carbon source such as [<sup>14</sup>C]-acetate is exhaled as <sup>14</sup>CO<sub>2</sub> within 24 hours. To estimate total caloric utilization of polydextrose, the actually recovered <sup>14</sup>CO<sub>2</sub> is corrected by the catabolic conversion factor (acetate to CO<sub>2</sub>) of 0.6. <sup>†</sup>Caloric utilization was determined over a 24-hour collection period. <sup>‡</sup>Caloric utilization was determined over a 13-hour collection period. NR=not reported.

Source: Figdor and Rennhard (1981)

Figdor and Rennhard (1981) also investigated polydextrose excretion in rats administered unlabeled polydextrose for 90 days. Male rats were fed *ad libitum* either a basal diet (i.e., control) or a treated diet supplemented with polydextrose (corresponding to approximately 1 or

10 g/kg bw/day). After 90 days of dietary exposure, both control and treated rats (460-650 g) were gavaged with 30 mg/kg bw of [ $^{14}\text{C}$ ]polydextrose (36.7  $\mu\text{Ci}$ ) in 1 ml solution, then placed in a metabolism chamber in which exhaled  $^{14}\text{CO}_2$  (every hour for the first 13 hours), feces and urine (at 24, 48 and 72 hours) were collected. The percentage of polydextrose excreted in air and urine did not differ among the three groups of rats (Table 7). The percentage of polydextrose eliminated in the feces was similar between the control group and 1 g/kg bw polydextrose group, and somewhat lower in the 10 g/kg bw polydextrose group. Figdor and Rennhard explained the reduced polydextrose fecal excretion in the high dose group to be the result of collection problems because the fecal matter of this dose group was soft and smeared onto the fur of the rat, preventing total recovery of the material.

Table 7. Excretion of [ $^{14}\text{C}$ ]PDX Administered Orally to Male Rats for 90 Days

Dose	Polydextrose Dose (units are % of administered dose)		
	Control	1 g/kg bw	10 g/kg bw
Air	31.2	32.6	30.8
Urine	1.8	1.5	2.4
Feces	56.4	52.4	43.8
Total	89.4	86.5	77
Source: Figdor and Rennhard (1981)			

In this study, all of the administered polydextrose was accounted for in expired air, feces and urine; thus, polydextrose is not stored in the tissues but eliminated from the body. The amount excreted in flatus specifically was not assessed. Although traditional methodologies for detailed assessments of pharmacokinetics and tissue distribution were not employed, the estimates of these parameters (elimination half-life of 30 minutes and distribution confined to extracellular space) are reasonable and logical. Importantly, Figdor and Rennhard (1981) reported that the only effect of administering 10 g/kg bw polydextrose to rats was the occurrence of soft stools; no adverse effects such as diarrhea were observed.

Juhr and Franke (1992) investigated the disposition of polydextrose in germ-free or conventional male Wistar rats (100-120 days old). Rats (n=8 per group) were fed a basal diet supplemented with 1% polydextrose for 2 weeks. Then each rat was gavaged with 359 kBq of [ $^{14}\text{C}$ ]polydextrose and immediately placed in a metabolism chamber for 30 hours. Expired air, urine and feces were separated, collected and analyzed for radioactivity. Two additional groups of rats (n=2 per group) were included that had their ceca removed surgically prior to treatment. Table 8 presents the disposition of [ $^{14}\text{C}$ ]polydextrose in germ-free (intact ceca), germ-free (cecectomized), conventional (intact ceca) and conventional (cecectomized) rats. All of the administered polydextrose was accounted for in expired air, urine, feces or carcass. In all four groups of rats, the primary route of excretion was feces (54%-81% of the administered dose). Approximately 20% more of the dose was excreted in the feces of germ-free rats compared to their conventional counterparts, indicating that some microbial degradation occurs within the large intestine, involving conversion to  $\text{CO}_2$  or volatile fatty acids and subsequent absorption. Approximately 4% was excreted in the urine (urinary constituents were not identified). The

percentage of polydextrose in expired air differed considerably between germ-free (11%) and conventional (30%) rats. Cecectomy did not appreciably affect the percentage of polydextrose in expired air. The higher percentage of polydextrose (~20% difference) in the expired air of conventional rats, compared to that in germ-free rats, indicates that a significant portion of expired  $^{14}\text{CO}_2$  is derived from the lower gastrointestinal tract. Cecectomy did not alter the disposition of polydextrose in germ-free rats; however, cecectomy reduced the percentage of polydextrose in the carcass (50%) of conventional rats, indicating that significant fermentation and uptake into the body occurs in the cecum.

**Table 8. Disposition of [ $^{14}\text{C}$ ]PDX Administered to Germ-Free and Conventional Male Wistar rats**

Locus	Germ-Free (% of administered dose)		Conventional (% of administered dose)	
	Cecum Intact (n=8)	Cecectomized (n=2)	Cecum Intact (n=8)	Cecectomized (n=2)
Expired Air	11.22±2.24	11.85±0.31	31.90±2.49	29.52±4.48
Urine	4.76±1.68	3.29±1.54	4.00±0.84	3.68±0.74
Feces	75.15±6.34	81.12±5.46	53.51±5.52	65.57±1.69
Carcass	9.64±3.60	7.94±2.90	12.81±2.15	6.31±3.00
Total	101.05±5.06	104.21±6.50	102.22±5.33	105.08±3.95
Source: Juhr and Franke (1992)				

The results of this study demonstrate that an appreciable portion (~30%) of ingested polydextrose is digested/fermented within the gastrointestinal tract and excreted in expired air. Juhr and Franke (1992) estimated that 12% of the administered dose is digested by gastrointestinal enzymes and approximately 18% is fermented within the lower gastrointestinal tract. These results are in agreement with the findings of Figdor and Rennhard (1981) and Cooley and Livesey (1987). In an *in vitro* study, Kruger et al. (1990) reported that the percentage of glucose liberated from polydextrose by small intestinal homogenate is only 5-8%.

## 2. Humans

Figdor and Rennhard (1983) investigated the absorption, distribution and excretion of polydextrose in human volunteers. Daily for 7 days, four male volunteers (age and body weights not reported) ingested non-labeled polydextrose (10 g or 0.17 g/kg bw/day; assuming body weight of 60 kg) dissolved in chocolate milk consumed immediately after breakfast. On day 8, the subjects ingested the drink containing non-labeled polydextrose plus 72  $\mu\text{Ci}$  of [ $^{14}\text{C}$ ]polydextrose. The subjects continued to receive non-labeled polydextrose on days 9 and 10, but not the radioactive polydextrose. Expired air was collected prior to receiving the drink supplemented with [ $^{14}\text{C}$ ]polydextrose, and at 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, 24, 36 and 48 hours following administration of radioactive polydextrose. All urine samples were collected and pooled between 0-6 hours, 6-12 hours and 12-24 hours on day one of [ $^{14}\text{C}$ ]polydextrose administration. Urine was subsequently collected and pooled over each 24-hour period for the next 6 days (total collection period was 7 days). All fecal matter was collected and frozen for 7 days after [ $^{14}\text{C}$ ]polydextrose administration. The amount of radioactivity (expressed as a

percentage of the administered dose) was determined in collected expired air, urine and feces. The quantity of volatile fatty acids excreted in human feces was also determined.

Approximately 40% of the administered polydextrose was excreted in the feces within the first 48 hours, and 50% was excreted in the feces by the end of the 7-day collection period (Table 9). Of the 50% that appeared in the feces, approximately 48% was unchanged polydextrose, while 2% was as volatile fatty acids. Twenty seven percent was excreted in the expired air as CO<sub>2</sub>. According to Figdor and Rennhard (1983), the CO<sub>2</sub> was derived from gastrointestinal absorption, as well as from absorption of small organic molecules that were produced by gastrointestinal microbes and degraded by mammalian enzymes into CO<sub>2</sub>. It is likely that the latter source (mammalian degradation of small organic molecules) contributed a significant amount of CO<sub>2</sub> to expired air because Figdor and Rennhard demonstrated *in vitro* that human fecal microbiota can convert a substantial amount (17%) of polydextrose to volatile fatty acids. An insignificant amount (1.4% of the administered dose) of radioactivity was detected in the urine. Of the radioactivity excreted in urine, 0.03% was unchanged polydextrose. Figdor and Rennhard (1983) postulated that the remaining urinary radioactivity was urea. As can be seen in Table 9, approximately 80% of the administered dose was recovered. Figdor and Rennhard proposed that the remaining 20% was lost as CO<sub>2</sub> in expired flatus, based on studies in humans in which it was demonstrated that another poorly digested carbohydrate, stachyose (a tetrasaccharide), increases CO<sub>2</sub> concentration of expired flatus up to 50% (Steggerda 1968).

Table 9. Excretion of [<sup>14</sup>C]PDX Administered Orally to Male Volunteers

Collection interval	Radioactivity as Percent (%) of Administered Dose				
	0-24 hr	24-48 hr	48-72 hr	72-168 hr	Total
Caloric utilization	26.62±7 <sup>†</sup>		NM	NM	26.62±7
Urine	0.83±0.1	0.33±0.2	0.11±0.1	0.14±0.05	1.41±0.4
Feces	2.53±2.8	39.66±16.3	6.11±7.6	1.77±2.8	50.07±7.5
Total	NR	NR	NR	NR	78.09±2.5

<sup>†</sup>Caloric utilization was determined over a 24-hour collection period <sup>‡</sup>Caloric utilization was determined over a 13-hour collection period, NM=not measured, NR=not reported

Source: Figdor and Rennhard (1983)

The fate of polydextrose in humans is similar to that in rats, i.e., the major excretory route is the feces, with appreciable biodegradation to CO<sub>2</sub> and an insignificant amount excreted in the urine. Although not all administered radioactivity is accounted for, it is reasonable to suggest that the polydextrose unaccounted for is excreted in flatus. Figdor and Rennhard (1983) did not report any adverse effects in male subjects from consumption of 10 g or 0.17 g/kg bw/day polydextrose for seven consecutive days.

Achour et al. (1994) also investigated the fate of polydextrose in 7 male volunteers 25-29 years of age. Subjects had normal body weight (data not reported), no history of gastrointestinal disease, no recent treatment with antibiotic, had never taken a laxative and were non-methane producers. The study design included a control period (CP=days 1-8), a short-term polydextrose consumption period (PD1=days 9-16) and a longer-term polydextrose consumption period (PD2=days 17-38). During the PD1 and PD2 periods, subjects consumed 30 g of polydextrose

daily (10 g, 3 times/day at mealtime in fruit juice). A controlled diet was administered on days 1 to 16 (CP and PD1) and days 31 to 40 (PD2). Subjects were allowed to eat their usual diet on the other days of the experimental periods. On days 13 (PD1) and 35 (PD2), [ $^{14}\text{C}$ ]polydextrose (20  $\mu\text{Ci}$ ) was consumed with non-labeled polydextrose during the first meal. Urine and feces were collected on days 5-8 (CP), 13-16 (PD1) and 35-38 (PD2) and analyzed for radioactive content. On days 5, 13 and 35, expired air was collected hourly from 0 to 15 hr, and then at 18, 21, 24, 30, 36 and 48 hr. For three subjects, flatus was collected for 12 hr on days 13 and 35.

Consumption of 30 g of polydextrose during PD1 and PD2 periods had no effect on transit time through the gastrointestinal tract, fecal weight (dry or wet), bacterial mass or fecal water content. Table 10 presents the percentage of radioactivity recovered from expired air, urine and feces during the PD1 and PD2 periods. The percentage of radioactivity recovered from expired air and feces was approximately 30 and 36%, respectively. A small amount (~4%) was recovered from urine. Not all administered radioactivity was accounted for in this experiment. The investigators attributed this lack of recovery to (1) incomplete collection of the label and/or (2) overestimation of the conversion factor of acetate to  $\text{CO}_2$ . Total volume of flatus expired during the PD1 period was about twice the volume expired during the PD2. Expired gas was analyzed for nitrogen, oxygen, hydrogen, carbon monoxide and methane, and levels were not significantly different between PD1 and PD2 periods (data not shown).

**Table 10. Excretion of [ $^{14}\text{C}$ ]PDX Administered Orally to Male Volunteers**

Locus	Radioactivity as Percent (%) of Administered Dose	
	PD1 (13 days)	PD2 (35 days)
Expired Air	30.8 $\pm$ 4.5	29.2 $\pm$ 3.7
Urine	4.3 $\pm$ 1.2	3.8 $\pm$ 0.7
Feces	~36.1	35.4
PDX*	32.7 $\pm$ 3.1	31.6 $\pm$ 3.7
Bacteria	3.0 $\pm$ 0.6	3.6 $\pm$ 1.2
Volatile fatty acids	0.4 $\pm$ 0.3	0.2 $\pm$ 0.1
Total**	86 $\pm$ 8	81 $\pm$ 7

\*Total radioactivity in feces minus radioactivity in bacteria and volatile fatty acids

\*\*Total was calculated by subtracting from % radioactivity in expired air (3%  $\text{CO}_2$  from monomeric fraction plus 16.5%  $\text{CO}_2$  derived from bacteria) divided by a conversion factor of acetate to  $\text{CO}_2$  plus % in feces plus % in urine

Source: Achouret et al (1994)

The polydextrose used in this study contained 5% monomers and 95% polymers of glucose units. Achour et al. (1994) concluded that the monomers are absorbed in the small intestine; while the polymers are transported to the large intestine, where 62% is fermented (see Table 11). Fermentation results in degradation of polydextrose, the products of which are either taken up into bacterial biomass (3%), or converted to volatile fatty acids (VFA) and gas ( $\text{CO}_2$ ). The remaining 33% of polydextrose is excreted unchanged in feces.

Table 11. Metabolism of [<sup>14</sup>C]PDX Administered Orally to Male Volunteers

Locus	Radioactivity as Percent (%) of Administered Dose
Unchanged PDX (feces)	33
Fermentation products	62
Bacterial biomass	3
Volatile fatty acids and gas	59
Total	95
Source Achour et al (1994)	

The results of this study by Achour et al. (1994) are in agreement with the previous study by Figdor and Rennhard (1983). Both studies demonstrate that (1) significant amounts of ingested polydextrose are excreted in the feces unchanged, (2) the remaining polydextrose is fermented within the lower gastrointestinal tract to CO<sub>2</sub> and volatile fatty acids and excreted primarily in expired air, (3) an insignificant amount is excreted in the urine and (4) colonic microbiota do not adapt to repeated exposure to polydextrose. Because the duration of exposure to polydextrose was considerably longer in the study by Achour et al. (1994) and no adverse effects were reported, the data indicate that doses up to 30 grams (i.e., 0.5 g/kg bw/day; assuming body weight of 60 kg) of polydextrose are well tolerated.

The effect of polydextrose ingestion on breath hydrogen and methane production were investigated by Kondo and Nakae (1996). Polydextrose (single administration of 7 g polydextrose/person/day) was administered in a beverage to men and women. The investigators found that polydextrose increased breath H<sub>2</sub> and CH<sub>4</sub> excretion only a small amount after about 1-2 hours, suggesting that the production of short chain fatty acids as a source of energy was low. The increases were significantly less than were produced by fructooligosaccharide or galactooligosaccharide. In another study, Solomons and Rosenthal (1985) investigated hydrogen gas production as an index of bacterial fermentation. Oral ingestion of 15 g polydextrose by healthy volunteers did not increase breath H<sub>2</sub>. In an *in vitro* study, Mazur et al. (1993) demonstrated that compared to other soluble carbohydrates, polydextrose is slowly fermented.

### 3. Summary of ADME Studies

The disposition of polydextrose is similar between rats and humans. Figure 3 illustrates the disposition of orally ingested polydextrose *in vivo*. Polydextrose is not stored within tissues but, rather, is completely excreted in feces, expired air or urine. Only a minor amount (approximately 12% in rats) of polydextrose is digested by gastrointestinal mammalian enzymes within the small intestine (Radosta et al. 1992). Oku et al. (1991) reported that polydextrose has a few α(1-4) and/or α(1-6) glucose linkages that can be hydrolyzed by the small-intestinal digestive enzyme maltase. The degradation of polydextrose by rat jejunal enzymes (mucosal homogenates) has been investigated *in vitro* by Ziese et al. (1995), who demonstrated that 8% of 2.5 mg/ml polydextrose was degraded and that this degradation could be inhibited by an α-glucosidase inhibitor (acarabose). Richter et al. (1994) reported that the *in vitro* digestibility of polydextrose by gastrointestinal mammalian enzymes is 22.5% and that this digestion results in its complete hydrolysis. Within the large intestine, polydextrose is subjected to degradation by

resident microbiota to CO<sub>2</sub> and volatile fatty acids (VFA), which are subsequently absorbed into the body. The CO<sub>2</sub> that is produced within the large intestine and absorbed is transported to the lungs and excreted in expired air. Volatile fatty acids that are absorbed from the lower gastrointestinal tract are further degraded by mammalian enzymes to CO<sub>2</sub> and excreted in expired air, while a small amount is incorporated into excretory products, such as urea, and are subsequently excreted in the urine. Lastly, a significant portion of an orally ingested dose of polydextrose is excreted in the feces unaltered by either mammalian digestive enzymes or bacterial fermentation processes.

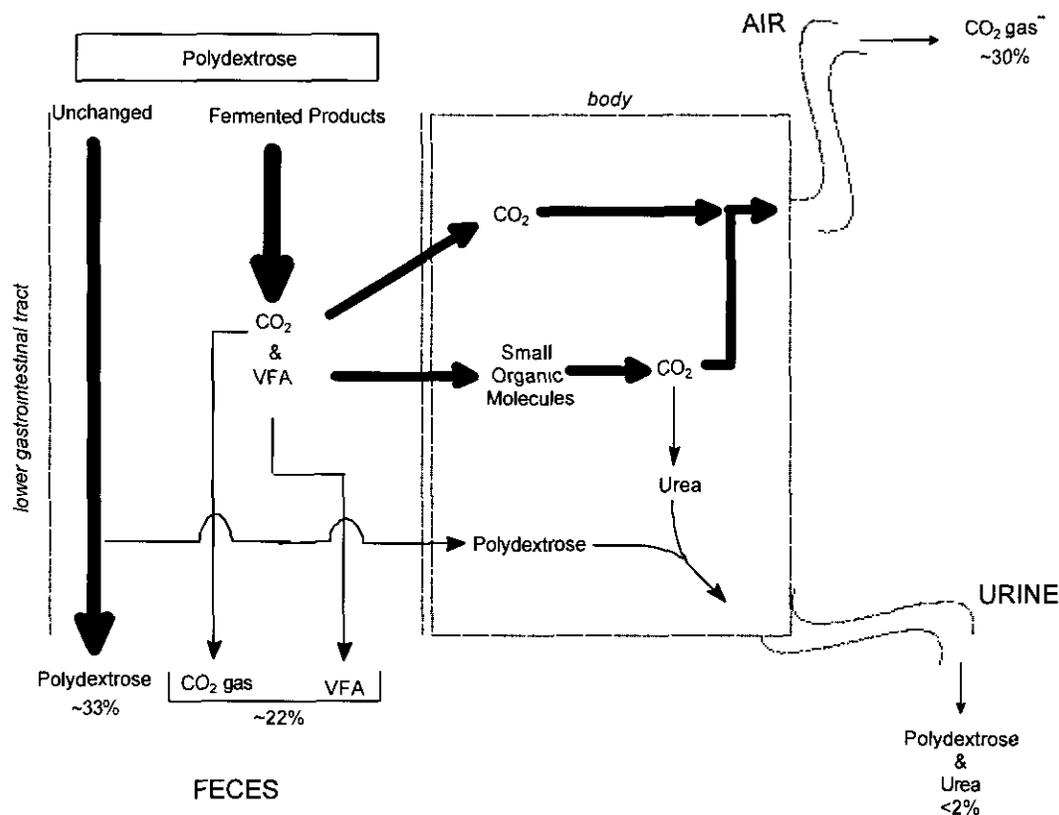


Figure 3. Absorption, Distribution, Metabolism and Excretion of polydextrose.

## C. Preclinical Studies

### 1. Biological Effects

The effects of polydextrose on the gastrointestinal tract have been investigated in rats and, as discussed below, include general physiology, microbiota, nutrient absorption and diarrhea. The authors of the studies that investigated gastrointestinal effects did not distinguish whether the polydextrose administered was the acidic or neutral forms.

### a. General Gastrointestinal Physiology

Bamba et al. (1993) investigated the effect of polydextrose on brush-border membrane enzymes of the small intestine in Wistar rats. Male Wistar rats (4 weeks old; n=6 per group) were fed a basal diet or the basal diet supplemented with 5% polydextrose for 2 or 4 weeks. After the experiment was terminated, the intestine from the pylorus to the ileocecal valve was removed. The small intestinal mucosa was scraped and maltase, sucrase and alkaline phosphatase activities were measured. Body weight was reduced 6% and 11% in rats fed 5% polydextrose for 2 or 4 weeks, respectively. Food intake was unaffected in rats fed polydextrose for 2 weeks, and reduced 16% in rats fed polydextrose for 4 weeks. Brush-border enzyme activity of the small intestine was unaffected by polydextrose treatment. The data indicate that polydextrose does not adversely affect small intestinal brush-border enzymes involved in digestion of complex carbohydrates.

Murai et al. (1994) investigated the effect of polydextrose on fecal pH, water content, short-chain fatty acids (SCFA) and the amount of  $\beta$ -glucosidase,  $\beta$ -glucuronidase, tryptophanase, and urease of Sprague-Dawley rats. Male rats (100 g body weight; n=5 per group) were fed either a basal diet or a basal diet supplemented with 13% polydextrose (approximately 20 g/kg bw) for 12 days. Fecal water content, pH, SCFA, and enzyme activities were measured on the treatment days 4, 5, 6 and 12. Fecal water content was increased 13% in polydextrose-treated rats. Fecal pH and SCFA were unchanged by polydextrose treatment. When measured at fecal pH (i.e., 6.19) the amount of  $\beta$ -glucosidase was increased 3.5-fold, while  $\beta$ -glucuronidase and tryptophanase were reduced 69% and 25%, respectively. Fecal urease activity was unaffected by polydextrose. Polydextrose was administered only for 12 days; this short treatment duration is a limitation in the study design. The effect of polydextrose on food consumption in this study is unknown because it was not reported. Because only one dose level of polydextrose was used in this study, it is unclear whether the effects on fecal enzymes were attributable to treatment. Further, because enzyme activity was not measured and the optimal pH of fecal enzymes differs between rats and humans, the significance of polydextrose on these fecal enzymes is equivocal.

Yoshioka et al. (1994) conducted two experiments in which the effect of polydextrose on the pH and morphology of the large intestine in Sprague-Dawley rats was investigated. In the first experiment, male rats (128-144 g body weight; n=15-16 per group) were fed a diet supplemented with 5% or 10% (i.e., 5 or 10 g/kg bw) polydextrose, cellulose, or galactomannan for 52 days. In the 10% groups, the levels of all three materials were gradually increased over a six-day period. On treatment day 53, rats were divided into two subgroups. In one group (n=10-11), rats were killed at 8:00 am (prior to eating) and cecal weight, surface area, crypt depth and muscular layer thickness and colonic weights and length were measured. In the second group (n=5), rats were killed at 11:00 am (after eating) and cecum (luminal pH, weight, surface area, crypt depth, muscular layer thickness were measured) and colon (luminal pH, crypt depth and muscular layer thickness were measured) parameters were measured. In the second group (n=5), rats were killed at 11:00 am (after eating) and cecum and colon parameters were measured.

Table 12 presents the results of Yoshioka et al. (1994) concerning the effect of polydextrose, cellulose and galactomannan on the cecum and large intestine of rats treated for 52 days. Food intake was slightly reduced by the higher concentration of polydextrose and to a lesser extent by galactomannan, though differences in body weight were not significant for any of the materials. Relative to the cellulose groups, both cecal weights and surface areas were higher in the polydextrose and galactomannan groups at both the 5% and 10% concentrations

and the values increased in a concentration-dependent manner. Constituents of rat cecal microbiota, including representative lactic acid bacteria (LAB) such as bifidobacteria and lactobacilli, are known to be stimulated by the inclusion of prebiotic materials such as raffinose (Dinoto et al. 2006). The growth of LAB and other bacteria capable of metabolizing polydextrose and galactomannan would reasonably be expected to produce lactic acid and additional short-chain fatty acids (SCFA), reducing the local pH in the process. Increased microbial biomass and subsequent nutrient exchange could contribute to increased cecal weight and this is supported by the observation that the cecal pH was reduced by both polydextrose and galactomannan.

The muscular layer of the cecum also varied with treatment and was thickest in the rats fed the cellulose diets and thinnest in the rats fed polydextrose (Yoshioka et al. 1994). These differences were significant in both the 5% and 10% addition conditions, but there was no dose-response effect as the thickness of the cecal muscular layer did not differ between 5% and 10% addition of any of the three supplements. With regard to the thickness of the colonic muscular layer, there were no differences among the rats receiving cellulose, polydextrose, or galactomannan at 5% of the diet. At the 10% addition, the rats receiving galactomannan had the thickest colonic muscle layers and those receiving polydextrose had the thinnest. Again, the 5% and 10% conditions were not significantly different, indicating no dose-response relationship.

**Table 12. Effect of Cellulose, PDX, and Galactomannan Fed to Rats for 52 Days on Cecum and Large Intestine Parameters**

Parameter	Cellulose (5%)	Polydextrose (5%)	Galactomannan (5%)	Cellulose (10%)	PDX (10%)	Galactomannan (10%)
Number of rats	10	10	10	10	11	11
Body weight (g)	444±7	432±4*	428±9	437±6	406±7	433±8
Food intake (g)	972±12 <sup>a</sup>	948±9 <sup>a</sup>	863±13 <sup>b</sup>	1008±16 <sup>a</sup>	905±16 <sup>b</sup>	833±18 <sup>b</sup>
Cecal weight (g)	0.60±0.03 <sup>b*</sup>	0.84±0.04 <sup>**</sup>	0.80±0.02 <sup>**</sup>	0.61±0.03 <sup>b</sup>	1.24±0.05 <sup>a</sup>	1.18±0.04 <sup>a</sup>
Cecal surface area (cm <sup>2</sup> )	18±1 <sup>b</sup>	24±1 <sup>**</sup>	22±1 <sup>**</sup>	17±1 <sup>c</sup>	43±2 <sup>a</sup>	35±1 <sup>b</sup>
Cecal weight/surface area (g/cm <sup>2</sup> )	35±1	35±1	36±1	36±1	30±1	34±1
Number of rats	5	5	5	5	5	5
Colonic weight (g)	1.6±0.1	1.6±0.2	1.7±0.1	1.9±0.1	1.5±0.2	1.7±0.1
Colonic length (cm)	20±1	19±0*	18±1*	20±1	22±1	21±1
Colonic weight/length (g/cm)	79±4	82±11	93±7	92±5	66±8	79±6
Cecal pH	6.6±0.1 <sup>a</sup>	6.1±0.1 <sup>b*</sup>	5.8±0.1 <sup>b*</sup>	6.3±0.1 <sup>a</sup>	5.8±0.1 <sup>b</sup>	5.2±0.1 <sup>c</sup>
Colonic pH	6.2±0.2	6.1±0.2	5.8±0.1	6.5±0.1 <sup>a</sup>	5.9±0.1 <sup>b</sup>	5.9±0.1 <sup>b</sup>
Cecal crypt depth (µm)	117±8	103±6	138±12	107±9	134±9	139±16
Cecal muscular layer thickness (µm)	119±8 <sup>a</sup>	69±7 <sup>b</sup>	96±13 <sup>ab</sup>	123±7 <sup>a</sup>	61±6 <sup>b</sup>	97±15 <sup>ab</sup>
Colonic crypt depth (µm)	153±19	110±3	141±14	161±21	102±4	135±13
Colonic muscular layer thickness (µm)	150±15	101±18	126±18	127±10 <sup>ab</sup>	77±9 <sup>b</sup>	159±21 <sup>a</sup>
Values are means±SEM. Superscripts show significant difference within 5% and 10% supplementation groups (P < 0.05). *Statistically significant difference between 5% and 10% supplementation of the same dietary fiber group (0 < 0.05). Source: Yoshioka et al. (1994).						

In the second experiment (Yoshioka et al. 1994), male rats (195-221 g body weight; n=13-14 per group) were fed either a fiber-free basal diet or the basal diet supplemented with 5%, 10% or 20% polydextrose (5, 10 or 20 g/kg bw) for 92 days. The level of polydextrose in the 10 and 20% groups was gradually increased over a 14 and 28 day period, respectively. Yoshioka et al. (1994) reported that rats were isoenergetically meal-fed and that 4.2 kJ/g was used as the energy value for polydextrose. On the 93<sup>rd</sup> day of treatment, rats were killed and cecum and large intestine parameters were measured. Table 13 presents the effect of polydextrose on cecum and large intestine when fed to rats for 92 days. Body weights were unaffected by polydextrose treatment. Cecal weights, surface area and pH were dose-dependently affected by polydextrose treatment. Cecal weight and surface area were increased in rats treated with 10% polydextrose

(130% and 200%, respectively) and 20% polydextrose (400% and 320%, respectively), while the lowest dose (5% polydextrose) had no effect on these parameters. Again, these changes could result from increased microbial activity and this hypothesis is supported by the observation that polydextrose treatment at all three doses reduced cecal pH by 0.4-0.5 pH units..

Table 13. Effect of PDX Fed to Rats for 92 Days on Cecum and Large Intestine Parameters

Parameter	Basal Diet	PDX (5%)	PDX (10%)	PDX (20%)
Number of rats	9	9	9	8
Body weight (g)	432±5	451±8	457±11	447±8
Cecal weight (g)	1.3±0.1	1.8±0.1	3.0±0.1*	5.3±0.9*
Cecal surface area (cm <sup>2</sup> )	15±1	22±1	46±3*	63±3*
Cecal weight/surface area (g/cm <sup>2</sup> )	86±3	83±5	68±5	84±14
Cecal pH	6.8±0.1	6.2±0.1*	5.9±0.1*	6.2±0.2*
Colonic pH	6.7±0.1	6.3±0.2*	6.3±0.0*	6.2±0.1*
Number of rats	4	4	4	6
Cecal crypt depth (µm)	131±7	136±9	159±15	185±19*
Cecal muscular layer thickness (µm)	181±24	117±15	89±15	284±187
Colonic crypt depth (µm)	84±11	81±9	77±6	78±14
Colonic muscular layer thickness (µm)	121±5	123±14	121±12	95±8

Values are means±SEM, \*Significantly different from cellulose-treated group at the same dose. Statistical analysis was the two-way ANOVA followed by the Tukey's posthoc test ( $p < 0.05$ )  
Source: Yoshioka et al (1994)

Yoshioka et al. (1994) reported that regression analysis indicated a correlation between pH and cecal growth (i.e., weight and surface area) in rats treated with polydextrose for 92 days. Based on this finding, Yoshioka et al. (1994) suggested that the lowering of the cecal pH is an important factor in stimulating intestinal mucosal growth. This finding is in agreement with previously published studies regarding the effects of high-fiber diets on rats (Jacobs and Lupton 1986; Lupton et al. 1988). On first analysis there were no significant differences in cecal muscular layer thickness among the four groups. After removal from the 20% polydextrose group of two animals with high levels of neutrophilic leukocytes (indicating inflammation), the remaining polydextrose-fed rats had thinner cecal muscular layers than did the rats fed a fiber-free diet. Again, there was no significant dose-response relationship across the three levels of polydextrose. There were no significant differences in the thickness of colonic muscular layers between rats fed fiber-free and polydextrose diets.

Although they offered no statistics, Yoshioka et al. (1994) reported that many neutrophilic leukocytes were in evidence in large intestine samples taken from rats consuming

the highest PDX dosage (20%). Two of eight rats were described as “not normal healthy rats...and as having inflammation of the large intestine.” As cited above, a 20% inclusion rate of PDX would be expected to have a significant impact on cecal and colonic microbiota. Excess production of specific metabolic end products during microbial growth (e.g., H<sub>2</sub>S, lactic acid) can also be associated with chronic inflammation. As an example, Nafday and coworkers (2005) demonstrated that the luminal administration to Sprague-Dawley rats of (300 mM) short chain fatty acids (SCFA), normal end products of the anaerobic fermentation of complex carbohydrates, resulted in mucosal injury. Interestingly, the severity of the colonic mucosal injury was maturation dependent, with higher injury scores occurring at postnatal ages 3 and 9 days, and minimal scores at day 23.

In order to further study the effects of dietary fiber on the muscular layer weight of the large intestine, Yoshioka et al. (1995) fed 80 male Sprague-Dawley rats (138 g body weight; n=16 per group) a basal diet supplemented with 7% cellulose (control group), 7% polydextrose, 7% guar gum (galactomannan), or 7% polydextrose with either kaolin or cellulose added at a rate of 10% w/w. Body weight gain, large intestinal weight, muscular weight of the large intestine, dry fecal weight, pH of the large intestinal contents and transit time were recorded/measured.

Body weight gain, pH (6.42-6.71) of the large intestinal contents and intestinal transit time were unaffected by polydextrose treatment (Table 14). While the diet with added polydextrose resulted in lower muscular weight of the large intestine than did either cellulose or guar gum, addition of either kaolin or cellulose obviated the effect, with cellulose addition the more effective of the two. Based on the finding that addition of either cellulose or kaolin to polydextrose prevented any thinning of the muscular layer of the large intestine, Yoshioka et al. (1995) concluded that the apparent effect of polydextrose on the muscular layer was actually due to the absence of bulk in the diet.

**Table 14. Effect of PDX, Cellulose, Guar Gum, and Combinations of PDX and Cellulose or Kaolin, on Body Weight and the Muscular and Total Weights of the Large Intestine in Rats**

	Dietary Groups (n = 8)				
	Cellulose	Guar Gum	Polydextrose	Polydextrose + Cellulose	Polydextrose + Kaolin
Body Weight (g)					
1 Week	261±1*	251±4*	256±3*	258±2*	253±3*
5 Weeks	411±5 <sup>a</sup>	366±5 <sup>b</sup>	422±5 <sup>a</sup>	416±8 <sup>a</sup>	411±4 <sup>a</sup>
Large Intestinal Weight (g)					
1 Week	1.09±0.03 <sup>ab*</sup>	1.08±0.05 <sup>ab*</sup>	0.91±0.05 <sup>c*</sup>	1.00±0.03 <sup>abc*</sup>	0.96±0.06 <sup>b*</sup>
5 Weeks	1.29±0.05 <sup>a</sup>	1.27±0.03 <sup>a</sup>	1.10±0.05 <sup>b</sup>	1.24±0.05 <sup>a</sup>	1.11±0.02 <sup>b</sup>
Muscular Weight of the Large Intestine (g)					
1 Week	0.95±0.03 <sup>ab*</sup>	0.93±0.05 <sup>ab*</sup>	0.73±0.05 <sup>cd*</sup>	0.89±0.03 <sup>ab*</sup>	0.83±0.06 <sup>bc</sup>
5 Weeks	1.11±0.05 <sup>a</sup>	1.09±0.03 <sup>a</sup>	0.89±0.05 <sup>b</sup>	1.10±0.05 <sup>a</sup>	0.92±0.02 <sup>b</sup>
Values are mean ± SEM. Superscripts show significant difference among the five dietary groups (p<0.05). Statistically significant difference between rats fed the diets for 1 week and those for 5 weeks (p<0.05).					
Source: Yoshioka et al. (1995)					

In yet another study, Yoshioka et al. (1996) fed 6-week-old rats diets containing 7% added polydextrose, cellulose, or guar gum (galactomannan) for either 1 or 5 weeks. In both the 1-week and 5-week studies, there were no differences between the cellulose and polydextrose diets in weight gain or intestinal muscular weights, while the guar gum diet resulted in lower body weight but increased intestinal muscle weight.

To summarize the studies of Yoshioka et al. (1994, 1995, 1996), the thickness of the intestinal muscular layer (especially in the cecum) appeared to be affected by the dietary interventions of adding polydextrose, cellulose, or galactomannan (guar gum) to the diet at levels of 5% or more. However, the reported effects were inconsistent both within and across the studies. Equally important, no dose-response effect was seen in those studies in which multiple dosages were fed.

Since the addition of kaolin along with a source of soluble fiber negated the effect of the fiber alone, there is reason to believe that the bulk of the digesta played a role in the effect, although Yoshioka et al. (1994 1995 1996) suggested fermentability may also be a key determinant. Some light may be shed on this issue by a study by Tanabe et al. (2006) of the effect of indigestible dietary components on luminal mucin secretion. In this study, the authors concluded that the bulking effect was effective throughout the length of the large intestine while the effect of fermentability was limited to the cecum.

Burdock and Flamm (1999) reported a number of animal toxicity studies of polydextrose that included necropsies in which any effects of polydextrose on the weights of organs, including intestines, were assessed. Such assessments, of course, did not specifically measure the weight of the muscular layers, but would be expected to have detected substantial effects on muscular weight. Goodland and Wright (1983), discussed below, suggested that differences in intestinal weight are due primarily to differences in the weight of the muscular layers, suggesting that such changes likely would have been observed in the necropsies. In none of the studies reported in Burdock and Flamm (1999) were any effects on intestinal weights reported. Studies included:

- Male and female CD-1 mice received 0, 7.5, or 15 g/kg bw/day polydextrose for 528 days;
- Male and female SD rats received 0, 2, or 10 g/kg bw/day polydextrose for 92 days;
- Male and female SD rats received 0, 5, or 10 g/kg bw/day polydextrose for 704 days; and
- Male and female beagle dogs received 0, 2, 4, or 8 g/kg bw/day polydextrose for 760 days.

A number of other studies have examined the effect of diet on intestinal morphology, including muscular layers. Savory and Gentle (1976) fed adult Japanese quail for 4 weeks on either standard (low-fiber) diet or a diet with 20% oak sawdust as a source of insoluble fiber. The latter diet resulted in increased gut size, and the authors concluded that this was a reflection of variations in food intake and bulk rather than an effect of fiber per se.

Sakata (1986) fed rats with ileal fistulas an elemental diet with or without 10% kaolin and also provided either a mixture of short-chain fatty acids or a saline solution via the fistula for 14 days. The addition of kaolin—inert bulk—resulted in greater muscular development of the digestive tract, supporting Savory and Gentle's (1976) suggestion that thickening of the muscular layer resulted from increased bulk.

Goodland and Wright (1983) fed 2-3-month old male Balb-c mice either pellets, elemental diet, or elemental diet with added kaolin or cellulose. Muscular atrophy occurred in the

distal third of the small intestine and muscular layers were thinner in all except the pellet-fed rats. The loss of muscle was greatest in the group receiving the elemental diet alone and least in the group receiving cellulose supplementation of the elemental diet. The authors concluded that the epithelial and muscle layers of the colon can change independently and that inert bulk can stimulate the muscle.

Starck and Rahmaan (2003) fed adult male Japanese quail either a standard diet or a high-fiber diet (40% non-digestible fiber) for either 2 or 4 weeks and found that the high-fiber diet resulted in increased thickness of the intestinal muscular layer as well as such other effects as increased gizzard size, intestine length, mucosal surface, and vascularization of the mucosa. They concluded that, "Increased muscle layer thickness is certainly related to increased bulk."

Jacobs and Schneeman (1981) fed male Wistar rats a fiber-free diet or the same diet plus 20% wheat bran, finding that the addition of wheat bran resulted in a highly significant increase in the thickness of the colonic muscular layer.

Yasar and Forbes (1999) fed 7-day old broiler chicks air-dry mash with or without added water for 35 days. The mash with added water produced greater development of tissue glands of the proventriculus and gizzard, associated with reduced thickness of the muscular layer of these segments. It was suggested that this resulted from decreased viscosity of the mash. The effects were seen primarily in the cecum and colon, less in the duodenum or in the small intestine.

Marcal Natali et al. (2005) fed adult male Wistar rats either normoproteic (22% protein) or hypoproteic (8%) diets and found that protein restriction resulted in larger mucosa and smaller muscular layers in the duodenum. The authors saw this as adaptive changes in morphology to the nutritional condition. This finding also suggests that experimental diets that result in lower intake or absorption of protein may result in thinner intestinal muscular layers, and that this result is likely to be a result of the dietary distortion that results when a substance is tested with exaggerated doses rather than an effect of the substance itself.

In a similar study, Schoffen et al. (2005) also compared the effects of hypoproteic and normoproteic diets in male Wistar rats. In this study, although the animals receiving the hypoproteic diet had thinner intestinal muscular layers than those receiving the normoproteic diet the effect was not statistically significant.

It is clear from studies in a variety of species that there are many factors that influence both mucosal and muscular characteristics of the small and large intestines. These factors are not well understood, and it is not clear to what extent the changes are adaptive to the nutritional conditions and to what extent they are passive responses to changes in water content, bulk, fermentability, viscosity, or other attributes of the digesta. All of these factors, along with the protein content of the diet, have been suggested as influencing changes in the thickness and weight of the muscular layers of the large intestine or in specific regions of the intestine such as the cecum or colon.

Further, there is no indication in any of the published research that these morphological changes are in any way harmful or non-reversible. Rather, they seem to be temporary accommodations to differences in the amount of bulk in the diet as well as other factors. Finally, it is to be noted that significant differences in intestinal muscular layer thickness result only from substantial changes in the bulk content, fiber content, or viscosity of the diet. Since the addition

of polydextrose to infant formula will not result in a dietary concentration higher than 0.2%, there is a reasonable certainty that no adverse changes will occur in the intestinal morphology of the infant as a result of this addition.

Harada and coworkers (1995) investigated the potential impact of consumption of polydextrose or pectin on several markers of gastrointestinal development (maturation) during the weaning period using a suckling rat model. On postnatal day 10 Wistar Imamichi strain rats were divided into four groups and treated with pectin (0.25 mg/g bwt), polydextrose (2 mg or 4 mg/g bwt) or saline (control) by orogastric instillation twice daily at 12 hour intervals for 7 days. At the end of the feeding period, each pup was infused with a macromolecular marker solution (50 mg/ml bovine immunoglobulin G in saline) via a polyethylene cannula passed into the stomach, incubated in a warm cage for four hours, bled by cardiac puncture under anesthesia, and the plasma separated by centrifugation. The small intestine, cecum, and large intestine and pancreas were removed and weighed. Histological sections prepared of the proximal small intestine were frozen for subsequent morphological investigation.

At 16 days of age both small intestine length and wet cecal weight (including contents) were significantly increased by PDX (4 mg/g bwt/day) and pectin (0.5 mg/g bwt/day), though to a greater extent by the former. Though lactase activity was unaffected by fiber, a dose-dependent increase in maltase activity was detected in the proximal small intestine following PDX treatment. Only a minor increase was observed with pectin treatment.

Four hours after infusion, the plasma concentration of bovine IgG was highest in the control pups and reduced 74% and 90% in those receiving low (2 mg/g bwt) and high (4 mg/g bwt) doses of PDX, respectively. The authors failed to highlight a 25% reduction (point estimates) with pectin at a dosage of 0.25 mg/g bwt. The authors reported using a smaller pectin dose due to viscosity and even at a relatively low dose the material had an effect, suggesting that the activity is not unique to PDX. It is of interest to note that the authors cited viscosity as important factor in the activity of soluble fibers but then did not control the factor in their assessment.

In addition, the authors reported that PDX treatment was associated with increased pancreatic enzyme activity and a reduction in the concentration of absorptive epithelial cells containing endocytotic vesicles in the jejunum. Unfortunately, they did not take similar measures in the pectin treated group.

Following the observation that orally administered polydextrose induced intestinal closure of macromolecular transmission and precocious increase in maltase and pancreatic enzyme activity, the authors concluded that the oligosaccharide induced precocious maturation of the small intestine and exocrine pancreas in the suckling rat. Based on lesser effects observed with the significantly lower dose of pectin, and a lack of comparable measurements, the possibility of similar activity cannot be excluded.

At least three major factors have been shown to influence intestinal maturation including: 1) an intrinsic timing mechanism (coordinates transition to adult phenotype); 2) circulating hormones (i.e. glucocorticoids); and 3) extrinsic factors such as the luminal microbiota (Nanthakumar et al. 2005). The impact of the latter two factors on the intestinal maturation of Black Swiss mice was recently evaluated by Nanthakumar and coworkers. Litters of suckling mice were randomly divided and treated with a single subcutaneous dose of cortisone acetate

suspended in normal saline (Control) and maintained with their dams until sacrifice at 14 days. Germ free (GF) mice of the same strain were maintained under GF conditions for four weeks and subsequently inoculated with the intestinal contents of age-matched conventional (CONV) mice. They, along with age-matched GF and CONV mice were sacrificed two weeks later and the small intestines harvested, washed, and divided into duodenum, jejunum and ileum sections and assayed for trehalase (a marker of general gut development) and  $\beta$ 1,4-galactosyltransferase ( $\beta$ GT) (a marker of glycosyltransferase development) gene expression.

In mammals, bacterial colonization begins at birth and adherence is mediated in part through interactions with glycoconjugates on brush border membranes. Bacterial colonization has been shown to regulate the activity of host glycosyltransferases which leads to changes in intestinal surface carbohydrates. As the  $\beta$ GT enzyme is involved in the synthesis of the glycan core (and not the more variable carbohydrate terminus) it was assayed as a potential marker of developmental variation in general glycan expression. Trehalase, which is involved in the digestion of Glc a1,6 Glc from branched chain dietary glucans, was selected as a potential marker for enterocyte development.

In CONV mice, both  $\beta$ GT and trehalase gene expression increased to adult levels by the fourth postnatal week (Nanthakumar et al. 2005). In contrast,  $\beta$ GT expression remained at a low levels in GF mice until stimulated by the introduction of adult luminal microbes (but not the microbiota of suckling mice). Comparable developmental patterns in colonic galactosyl  $\beta$ 1,4-linked glycoconjugates (the product  $\beta$ GT activity) were observed. These results indicated that the normal development of an adult pattern of glycosyltransferase expression depended upon the presence of microbiota that were typical of the adult gut, suggesting an essential role for specific bacterial colonization in intestinal mucosal cell surface glycoconjugate receptor ontogeny.

Butyrate short-chain fatty acids (SCFA), are one of the primary end products resulting from the anaerobic bacterial fermentation of complex carbohydrates (including prebiotic oligosaccharides) in the gastrointestinal tracts of humans and other mammals. Efficient metabolism of PDX to SCFA (including butyrate) by human colonic bacteria has been demonstrated using a three-stage, continuous culture system (Probert et al. 2004). Concurrently, PDX had a stimulatory effect on the colonic bifidobacteria populations at concentrations of 1% and 2%. Butyrate has also been identified as the preferred energy source for the colonic mucosa and is thought to play a largely protective role in gastrointestinal health (reviewed by Pryde et al. 2002).

Kotunia and coworkers (2004) recently investigated the effects of butyrate on small intestinal development in neonatal piglets fed by an artificial sow. In the study 3 day old piglets were fed for 7 days with an artificial milk formula alone (Control) or the control formula supplemented with sodium butyrate (0.3 g/100 g dry matter). At 10 days of life, the piglets were sacrificed and whole-thickness samples of the duodenum, jejunum and ileum were fixed for histological analysis. In addition, blood and mucosal scrapings were collected for plasma gastrin, cholecystinin (CCK) and pancreatic polypeptide (PP) and intestinal brush border enzyme activities, respectively. Sodium butyrate supplementation resulted in increased crypt depth, villi length and mucosa thickness in the distal jejunum and ileum. While no effect was observed on intestinal brush border enzyme activity, plasma CCK and PP were increased. The authors concluded that sodium butyrate supplementation contributed to intestinal mucosa development in neonatal piglets.

It is increasingly recognized that postnatal gut maturation is partially modulated by the bacterial colonization process. This hypothesis was recently evaluated by Schumann et al. (2005) by treating suckling Sprague-Dawley rats with daily administration of a broad-spectrum antibiotic (Clamoxyl, a commercial preparation of amoxicillin) from postnatal day 7 until day 17 or 21. The proximal and distal small intestine and colon were removed and frozen for microarray analysis of gene expression after their luminal contents were removed in ice-cold sterile saline. Clamoxyl treatment drastically reduced total aerobic and anaerobic bacteria and almost completely eradicated lactobacilli from the whole intestine. A significant portion of the genes undergoing maturation (10-30% depending upon location) were modulated by Clamoxyl treatment. Downregulation of Paneth cell products (defensins, matrilysin) and major histocompatibility complex (MHC) class Ib and II genes (involved in antigen presentation) was also observed.

A different research group demonstrated that Paneth cells (a key component of the innate immune system) were strategically positioned to coordinate the development of the underlying vasculature of intestinal villi and the normal microbiota (Stappenbeck et al. 2002). Taken together, these results suggest that early treatment with antibiotics (with the concomitant impact on the resident microbiota) has the potential to negatively influence gut barrier function at the critical suckling-weaning interface.

The early intestinal maturation (closure) in suckling rats that was observed by Harada et al. (1995) that occurred in the polydextrose treated groups could be attributed to changes in the resident microbiota and their metabolic end products. Intestinal maturation is known to be influenced by multiple factors including the luminal microbiota. Changes in intestinal maturation have been demonstrated in studies where normal microbiota were either supplied to germ-free animals or radically altered via broad-spectrum antibiotic treatment. Further studies have shown that the potential impact of microbiota on intestinal maturation could be mediated through simple metabolic end products such as butyrate, one of the SCFA produced during the anaerobic fermentation of polydextrose and other complex polysaccharides.

An additional interpretation of the results of Harada et al. (1995) is that early intestinal maturation may represent a physiological mechanism by which prebiotic oligosaccharides decrease the risk of infection and/or food intolerance (allergy) via the exclusion of pathogenic microorganisms and dietary antigens, respectively. This is certainly plausible as breast feeding has long been associated with such protective effects, and breast milk is known to contain significant quantities of a range of oligosaccharides (actually the third largest component). This is in line with the rationale of adding PDX to infant formula to mimic the performance of human milk oligosaccharides.

In summary, the changes in intestinal morphology observed by Yoshioka and by Harada are not unique to polydextrose but rather occur in multiple species with other fermentable bulk substances and thus appear to be generalized effects of prebiotics. Additionally, there is no reason to regard them as adverse but rather as adaptive and potentially protective and thus arguably beneficial.

#### **b. Gastrointestinal Micro biota**

Sugawa-Katayama (1994) investigated the effect of polydextrose on cecal microbiota of three-week old male Sprague-Dawley rats. Rats (n = 4) were fed a basal diet supplemented with 5% cellulose (control) or 10% polydextrose (6.8 g/kg bw) for 4 weeks. After the four-week

treatment period, body weights (g), food intake (g/day), feces excreted (g/day), cecal pH, cecal content (g) and cecal microbiota (Enterobacteriaceae, Streptococcus, Staphylococcus, Lactobacillus, Bifidobacterium, Eubacterium, Bacteroidaceae, Peptococcaceae and Clostridium) were measured. Mean body weights, body weight gain and food intake were unaffected by polydextrose treatment. Polydextrose feeding resulted in diarrhea during the first three days, soft stools after one week and solid stools after three weeks of feeding. Cecal pH of polydextrose-treated rats was decreased compared to the control group. Cecal content was increased 167% in rats treated with polydextrose. Of the nine groups of microbiota examined, three were found to be elevated above control rats and included Enterobacteriaceae ( $7.7 \pm 0.6$  polydextrose versus  $6.8 \pm 0.2$  control), Streptococcus ( $8.1 \pm 0.2$  polydextrose versus  $5.9 \pm 0.4$  control) and Peptococcaceae ( $9.7 \pm 0.3$  versus  $8.8 \pm 0.5$ ). The investigators did not provide any interpretations of these increases. The other six species of microbiota were unaffected by polydextrose.

### c. Nutrient Absorption

In the following sections, the effect of polydextrose on gastrointestinal absorption processes, and the absorption of glucose, calcium and lipids in rats is critically evaluated.

#### (1) Transport Processes

Yoshioka et al. (1996) investigated the effect of polydextrose on absorptive processes (i.e., passive carrier-mediated transport rate, active carrier-mediated transport rate, and intracellular permeation rate) in Sprague-Dawley rats. Male rats ( $n=16$  per group) were fed a non-purified diet supplemented with 7% polydextrose (12 g/kg bw) for 1 or 5 weeks. The control group was fed the non-purified diet supplemented with 7% cellulose. On the final day of the experiment, body weights were recorded, animals were killed and the small intestine was removed, weighed and analyzed for absorption rate. Absorption rate was measured using the everted sac technique. Data were analyzed for statistically significant differences by ANOVA and Tukey's post-hoc test. Body weights, small intestinal weights and small intestinal mucosal and muscular weights were unaffected by polydextrose treatment. After 1 week of treatment with polydextrose, *in vitro* measurements of passive carrier-mediated transport, active carrier-mediated transport and intracellular permeation rates were reduced up to 90%. After 5 weeks of treatment, all three absorption processes were unaffected in rats treated with polydextrose. The data indicate that, although initial absorption processes decrease in polydextrose-treated rats, the rat gastrointestinal tract adapts and restores the absorptive function to normal capacity.

#### (2) Glucose

Glucose absorption from the gastrointestinal tract in rats is reported to be unaffected by polydextrose (Fuse et al. 1991; Ogata et al. 1997). Ogata et al. (1997) is critically evaluated on page 34.

#### (3) Calcium

Hara et al. (2000) investigated the effect of polydextrose on calcium absorption *in vivo* and *in vitro*. In the *in vivo* experiment, normal or gastrectomized male Sprague-Dawley rats ( $n=22$  and  $21$ , respectively) were fed polydextrose (5% dietary concentration or approximately 2 g/kg bw) for 21 days. Calcium (3.0 g Ca/kg diet) was supplied as  $\text{CaCO}_3$  (water-insoluble salt). Vitamin  $\text{B}_{12}$  and  $\text{FeCl}_2$  were administered subcutaneously every 5 days. Body weights and food consumption were measured daily. Feces were collected continuously for the last 3 days for evaluation of calcium excretion and apparent calcium absorption. The study was terminated by killing the rats and removing the right femur and cecum (with contents). For the *in vitro*

experiment, everted sacs of the jejunum and ileum (i.e., small intestine) were bathed in an artificial mucosal fluid containing polydextrose (50 g/liter) and calcium (10 mM  $\text{CaCl}_2$ ). Artificial serosal fluid was passed through the sacs and analyzed for calcium.

In the *in vivo* study, mean body weights of polydextrose-treated normal and gastrectomized rats were increased 11% and 27% by the end of the 21-day treatment period. Mean food intake was unaffected by polydextrose treatment in either normal or gastrectomized rats. Mean fecal dry weights were unaffected by polydextrose fed to normal rats and increased 23% in gastrectomized rats fed polydextrose. Apparent absorption of calcium from the gastrointestinal tract was increased 8% and 100% in polydextrose fed normal and gastrectomized rats, respectively. Calcium absorption was significantly lower in control gastrectomized than normal control rats (15% versus 79% absorption, respectively). Mean femur weights were unaffected by polydextrose treatment in normal and gastrectomized rats. Mean femur calcium content was increased 5% and 10% in polydextrose-treated normal and gastrectomized rats, respectively. Mean weights of the cecal wall and cecal contents were increased by 68 and 237%, respectively, in normal rats treated with polydextrose; and increased in polydextrose-treated gastrectomized rats by 69 and 224%, respectively. Mean cecal pH was reduced in polydextrose treated normal rats (6.29 treated versus 7.26 control) and polydextrose-treated gastrectomized rats (6.06 treated versus 7.07 control). In the *in vitro* study, calcium absorption increased 42% in sacs obtained from the ileum. Calcium absorption in sacs obtained from the jejunum was unaffected by polydextrose *in vitro*.

In summary, the results from these *in vivo* and *in vitro* experiments indicate that polydextrose does not interfere with calcium absorption from the gastrointestinal tract, but rather, increases its absorption leading to increased calcium incorporation into bone, a beneficial effect.

#### (4) Lipid

Ogata et al. (1997) investigated the effect of polydextrose on lipid absorption in Sprague-Dawley rats. Male rats (2380-330 g) were infused with a lipid emulsion that contained 5% or 10% polydextrose (3 ml/hr for 8 hours). The total amount of polydextrose administered was 4 and 8 g/kg bw. The lipid emulsion contained 0.354 g of triolein, 1  $\mu\text{Ci}$  glycerol tri[9,10(n)- $^3\text{H}$ ]oleate, 0.051 g of cholesteryl oleate and 10  $\mu\text{Ci}$  [1- $^{14}\text{C}$ ]cholesteryl oleate. Total lymph flow, small intestine luminal volume, and lymphatic radioactivity output were measured. In rats infused with 10% polydextrose, lymph flow was reduced 35% compared to the vehicle control group, while small intestinal luminal volume was increased 62%. Lymphatic radioactivity output of triolein was reduced 19% and 32% in rats infused with 5% and 10% polydextrose, respectively. Lymphatic radioactivity output of cholesterol was reduced 10% and 15.9% in rats infused with 5% and 10% polydextrose, respectively. The percentage of infused triolein and cholesterol in the lumen of the gastrointestinal tract was dose-dependently increased in the proximal small intestine. The data indicate that polydextrose retarded lymphatic transport of triolein and cholesterol in the mesenteric lymph fistula rats, as well as inhibited lipid absorption. Ogata et al. also reported that glucose absorption was unaffected by polydextrose infusion.

Choi et al. (1998) investigated the effect of polydextrose on lipid metabolism in male Sprague-Dawley rats. Rats (n=8-10 per group) were fed a diet supplemented with 5% (w/w)  $\alpha$ -cellulose (control group) or 5% polydextrose for 6 weeks. After the treatment period, blood was sampled and the liver and small bowel were removed and analyzed for lipids. Jejunal maltase and sucrase activities were also measured. Body weight gain, feed efficiency and relative liver

weights were unaffected by polydextrose treatment. Plasma and hepatic total cholesterol, triglyceride and HDL cholesterol were also unaffected. Daily excretion of total lipids, cholesterol and bile acids were reduced 44%, 57% and 82%, respectively in rats whose diets were supplemented with polydextrose. The mechanism of increased excretion of lipids and bile acids may be related to increased water holding capacity (which slows gastric emptying) and an increased binding of bile acids, respectively. Maltase activity was unaffected by polydextrose treatment, while sucrase activity was reduced 31%. Choi et al. proposed that the reduced sucrase activity might contribute to normalization of serum glucose concentration.

#### **d. Laxation**

Laxation is a general term that refers to the normal process of defecation (elimination of fecal waste through the anus). While there is no strict definition for what constitutes normal laxation, the presence of softer, looser stools falls within a continuum of normal stool patterns. As an example, Stedman's Medical Dictionary refers to laxation as a bowel movement characterized by soft stools and reduced gastrointestinal transit time (Spraycar 1995e). The occurrence of a softer, looser stool pattern is typically reported for breast fed infants. When compared to formula fed infants, breast fed infants have been shown to pass more stools per day (median of 1.6 versus 4.4, respectively), the greatest range of stools per day (0.6-3.9 versus 0.3-8.0, respectively), as well as looser (runny, pasty) stools (Tham et al. 1996). This laxation (stooling) pattern could be attributed, in part, to the presence of significant quantities (5-10 g/L mature milk) of a large complex (> 130 structures) of human milk oligosaccharides (HMO) (reviewed by Bode 2006).

Even though a generally accepted definition of normal laxation is not available, constipation has been defined as "a lack of laxation and/or necessity of laxative therapy in at least 3 out of 6 days," (Meissner et al. 2000) and "difficulty in passing stools or an incomplete or infrequent passage of hard stools (Anderson 2003). When the term laxation is used in clinical literature, it is often in relationship to constipation, where the intended effect of a dietary or pharmaceutical intervention is the stimulation of a normal defecation pattern, or production of looser stools for more comfortable defecation.

It is important to distinguish diarrhea from laxation. Diarrhea is characterized by the presence of frequent, watery bowel movements accompanied by an excessive loss of fluid and electrolytes. The most widely accepted clinical definition of a diarrhea day is one where a subject "experiences three or more loose or watery stools in 24 hours or any number of loose or watery bloody stools," (Wright et al. 2006). Though the physiological basis for diarrhea is disturbed intestinal transport of solutes (primarily sodium, chloride and glucose) followed by passive water movement, several mechanisms, including secretory and osmotic abnormalities, have been identified as typical causative agents (Behrmen et al. 2004). Secretory diarrhea is characterized by watery, voluminous defecations, and is typically driven by the binding of a secretagogue (e.g. cholera toxin) to a receptor on the intestinal epithelium. Osmotic diarrhea is usually of lesser volume, stops with fasting and is often associated with the ingestion of a poorly absorbed solute (e.g. magnesium). In infants, acute diarrhea is more commonly associated with gastroenteritis, systemic infection and/or antibiotic use while the causes of chronic diarrhea are likely to include food intolerance (e.g. cow's milk, soy protein) or other disease states (e.g. cystic fibrosis, celiac disease).

Diarrhea then is an abnormal condition characterized by the frequent excretion of semisolid or fluid (i.e., watery) feces from the bowel (Spraycar 1995b). Thus, what distinguishes diarrhea from laxation is the frequency of fecal excretion and the watery consistency of the excreted feces as well as the loss of fluid and electrolytes. Laxation is generally not adverse to health; indeed, as has been noted, as compared with formula-fed infants, breast-fed infants generally exhibit a stooling pattern characterized by more frequent defecation, looser stools, and softer stools. Laxation effects that produce this pattern of stooling may thus be regarded as beneficial and clearly not adverse. Chronic diarrhea, on the other hand, can have deleterious health consequences when a nutritional deficit develops. Infants are particularly susceptible to the deleterious health effects of chronic diarrhea when the severity is sufficient to adversely impact nutritional intake and growth. It is not unexpected that substances that result in desirable laxation effects at certain levels of intake may produce diarrhea at higher levels; this ability to produce diarrhea at high levels of intake is not incompatible with the substance being regarded as safe under conditions of use that result in lower levels of intake.

Of the studies reporting a laxative effect by polydextrose in rats, there is some variation in the lowest dietary concentration that elicits the effect. For instance, Huang and Hsu (1996) reported that a purified diet supplemented with 5% polydextrose and fed to male Sprague-Dawley rats for 12 weeks resulted in diarrhea. Yoshioka et al. (1995) reported that the lowest dietary concentration that produced diarrhea in male Sprague-Dawley rats was 8%. In another study, Kanauchi et al. (1997) reported that a dietary concentration of 6% polydextrose fed to male Sprague-Dawley rats produced diarrhea. These slight differences in dietary concentrations are probably due to a combination of experimental factors, such as initial body weight of the rats and type of diet (purified versus non-purified). Although the investigators of these studies did not report the dose of polydextrose on a g/kg bw/day basis, it is estimated that the polydextrose dose used in these studies to produce diarrhea in rats was 10 g/kg bw/day or greater. Dow Corning (1994) reported that a daily diet of 15 g polydextrose (48 g/kg bw/day) fed to adult male Sprague-Dawley rats (n=5) for 7 days resulted in slight anal leakage (2 out of 5 rats) and oily feces (2 out of 5 rats). Treatment with polydextrose had no effect on body weight, body weight gain or food consumption. Because Yoshioka et al. (1995) demonstrated that a dietary polydextrose concentration below 8% did not elicit diarrhea in the rat, 10 g/kg bw/day is considered the lowest-observed-effect-level (LOEL).

Grossklaus et al. (1984) investigated the effect of sugar substitutes, including polydextrose, on the formation of short-chain fatty acids in the ceca of non-adapted and adapted juvenile rats (strain and gender were not reported). Rats were adapted by administration of 5% polydextrose in the drinking water ad-libitum for 12 days. Mean initial body weight of juvenile rats was 50.1 g, while mean body weight after adaptation was 102.5 g. In both non-adapted and adapted rats, polydextrose (1.5 or 3.0 g/kg bw/day) was administered intragastrically. One hundred and eighty minutes after polydextrose administration, cecal short-chain fatty acids (SCFA), water, pH and osmolality were measured. The SCFA were determined by gas chromatography in cecal water. Control rats for both non-adapted and adapted groups were fasted animals.

In non-adapted and adapted rats, cecal osmolality was unaffected by polydextrose treatment (Table 15) Cecal pH was reduced in non-adapted rats treated with the higher polydextrose dose (i.e., 3 g/kg bw/day), while pH was unaffected at the lower dose. (Cecal pH was reduced in rats treated with 1.5 or 3.0 g/kg bw/day polydextrose.) Cecal water content was increased in both non-adapted and adapted rats treated with 1.5 g/kg bw/day polydextrose (72%

and 58%, respectively) or 3 g/kg bw/day polydextrose (271% and 170%, respectively). In non-adapted rats, total SCFA was unaffected by the lower polydextrose dose and reduced 53% in rats treated at the higher dose. In adapted rats, total SCFA was unaffected by polydextrose treatment. Further investigation of specific SCFA in the non-adapted rats showed that acetic and propionic acids were reduced (42%-61% and 21%-41%, respectively) in rats treated with polydextrose (Table 16), while butyric acid was unaffected. In contrast to non-adapted rats, acetic and propionic acids were unaffected by polydextrose treatment in the adapted rats.

**Table 15. Effect of PDX on Cecal Parameters of Adapted and Non-Adapted Juvenile Rats**

Parameter	Non-Adapted (dose g/kg bw/d)			Adapted (dose g/kg bw/d)		
	Control	1.5	3	Control	1.5	3.0
pH	7.01±0.09	6.72±0.34	6.18±0.29*	7.02±0.24	6.4±0.25*	6.21±0.3*
Osmolality (mosm/kg)	395±40	434±120	389±38	387±64	397±30	425±41
Cecal water (ml)	0.195±0.07	0.335±0.106*	0.724±0.194*	0.277±0.057	0.438±0.167*	0.746±0.288*

\*Significantly different from control using the Wilcoxon test ( $p < 0.05$ )  
Source: Grossklaus et al. (1984)

**Table 16. Effect of PDX on Short-Chain Fatty Acids of Adapted and Non-Adapted Juvenile Rats**

Short-Chain Fatty Acids (mmole/l)	Non-Adapted (dose g/kg bw/d)			Adapted (dose g/kg bw/d)		
	Control	1.5	3	Control	1.5	3.0
Acetic acid	54.4±5.4	31.4±9.6*	21.4±8.9*	38.1±9.3	43.8±19.9	36.8±9.4
Propionic acid	13.9±1.8	11.0±2.0*	8.2±3.2*	11.1±3.3	12.9±4.6	8.8±1.7
Butyric acid	7.2±1.5	8.7±3.1	6.6±2.1	6.1±2.7	9.4±2.9	8.1±3.8

\*Significantly different from control using the Wilcoxon test ( $p < 0.05$ ). The SCFA were determined by gas chromatography in cecal water at 180 minutes after polydextrose administration.  
Source: Grossklaus et al. (1984)

The investigators reported a negative correlation between net cecal water content and total SCFA concentration. According to Grossklaus et al., the decline in cecal pH is an indicator of fermentation, while reduced SCFA is unequivocal evidence of fermentation. Thus, the reduction in SCFA concentration is due to a dilution effect. Grossklaus et al. concluded "the quantitative determination of the short-chain fatty acids in the cecum of the rat shows clearly that non-absorbed fractions of sugar substitutes can undergo belated metabolism by fermentation by the intestinal flora." Importantly, according to these investigators, "adaptation results in the cessation of initial diarrhea".

Lorenz and Grossklaus (1984) investigated the osmotic effect of various sugar substitutes, i.e., sucrose, sorbitol, palatinit<sup>®</sup>, lycasin<sup>®</sup> or polydextrose<sup>®</sup> (in electrolyte solutions) in juvenile rats orally dosed. Male Wistar rats (100 grams body weight) were fasted for 16 hours, then gavaged with 1.5 ml of 10% or 20% solution. A control group was included that was dosed with sugar-substitute-free electrolyte solution. After 15, 30, 60 and 180 minutes of test solution

administration, rats were anesthetized and blood sampled from the portal vein and analyzed for glucose concentration. The contents of the stomach, small intestine and cecum were collected and analyzed for net water movement. Net water movement in the gastrointestinal tract was dependent on the type of sugar substitute. Sorbitol was the most effective at increasing net water movement, whereas polydextrose was the least effective. Thus, polydextrose had the mildest osmotic effect as compared to the other sugar substitutes. Unlike the other sugar substitutes, polydextrose resulted in a lower rise in blood glucose. These data indicate that short-term administration of polydextrose produces less diarrhea in rats compared to other sugar substitutes.

#### **e. Summary of Biological Effects of Polydextrose in Rats**

The effect of polydextrose on the gastrointestinal tract has been extensively investigated in experimental animals, although as noted earlier, the experimenters in these studies did not indicate whether the polydextrose administered was the acidic or neutral form. The studies indicate that polydextrose has potential beneficial effects to the gastrointestinal system, such as increased calcium absorption, growth and neonatal maturation (Yoshioka et al. 1994; 1995; Hara et al. 2000). The latter beneficial effect, neonatal maturation, is of particular interest because it helps protect neonates from invasive external pathogens.

The effect of polydextrose on gastrointestinal absorption has been investigated in several studies, and the data indicate that polydextrose has minimal effect on gastrointestinal absorption. The reduced absorptive capacity within the first week of administration observed in rats treated with very high doses of polydextrose (12 g/kg bw or more) is a transient effect and absorptive capacity is fully restored by the 5<sup>th</sup> week of administration. In support of the lack of an appreciable effect on gastrointestinal absorption, it was demonstrated that polydextrose does not interfere with glucose absorption, and effectively enhances calcium absorption. The lack of an effect on absorption is in agreement with Osamu et al. (1990) and Huang et al. (1996).

The only potentially adverse effect of polydextrose to the gastrointestinal tract is diarrhea. The diarrhea effect is dose-dependent and the lowest effective dose level for inducing diarrhea in rats is estimated to be 10 g/kg bw/day. Importantly, it has been demonstrated that polydextrose does not adversely affect normal passive carrier-mediated and active carrier-mediated transport processes and does not interfere with intestinal absorption of sugars, minerals and lipids. Polydextrose also does not adversely affect gastrointestinal microbiota. The data indicate that even moderately high doses of polydextrose (i.e., up to 10 g/kg bw/day) do not directly produce an adverse effect on gastrointestinal function that impairs the health of rats.

## **2. Toxicity Studies**

Preclinical safety studies (conducted in bacteria, tissues and non-human species) are intended to identify and characterize potential hazards that might be elicited in humans when polydextrose is consumed in infant formula. These studies include both *in vitro* (usually cell cultures) and *in vivo* (whole animal) protocols. Between 1972 and 1979, Pfizer, Inc. conducted numerous preclinical safety studies on both forms of polydextrose (acidic and neutral). While these studies were submitted to FDA in support of a Food Additive Petition, they were not published in the scientific literature as individual studies. Rather, as is not uncommon with series of toxicity studies on a single substance, they were all published in a single article that presented a compendium of the preclinical research on polydextrose. This article, by Burdock and Flamm (1999), was published under the title, "A Review of the studies of the Safety of Polydextrose in Food." This title may have been unfortunate, in that "reviews" are generally considered as

background materials that lack sufficient information on individual studies to allow the scientific and regulatory communities to determine whether all critical elements are present from which scientific conclusions can be drawn. It is the opinion of MJ that this article is actually not a review at all, but rather a compilation of original research reports with a common discussion and conclusions. These individual reports were prepared by Burdock and Flamm based on their own analysis of the raw data from the toxicological laboratories, and not, as in a true review article, from summary reports prepared by others. In our opinion, each individual research study is reported at a level of detail consistent with that which would be available if the studies had been published individually. What Burdock and Flamm (1999) offers, in addition to detailed descriptions of the individual research studies, is a synthesis of the implications of the studies taken as a whole, a synthesis that could not have been provided if each study had been published individually. In this respect, the synthesis portion of the article does resemble a review, except that it integrates studies all published together in this single article rather than independently. It is probably for this reason that the authors chose to call the article a “review” rather than a “compendium” or something similar.

Our position that Burdock and Flamm (1999) is properly regarded as a compendium of studies rather than a review, and that it constitutes original publication of the toxicological research consistent with the requirement for GRAS status, is based on four considerations:

1. It does not meet the definition of a review article.
2. It is not formatted as a review article.
3. It presents analyses based on examination and analysis of raw data rather than summary reports.
4. It describes the research methods and results at a level of detail consistent with original publication rather than a review article.

We believe that these considerations, discussed more fully below, are sufficiently compelling that FDA can agree with our position without in any way compromising its stance that review articles alone are not sufficient to provide “general availability” of the information demonstrating general recognition of safety.

#### **Burdock and Flamm (1999) does not meet the definition of a review article.**

The term “review article” has a specific definition which is not met by the Burdock and Flamm article. Van Buskirk (1984), in an article discussing some ambiguities in National Library of Medicine patrons’ understanding of the term “review article,” quotes the MEDLARS Indexing Manual Part II as defining a “review” as a “review of the recent literature” or “review of the current literature.” Although some ambiguities remain (such as whether a “review of cases” or a history of research in an area constitutes a review), it is clear that a critical mandatory requisite for an article to be regarded as a review is that it must deal with *published* articles, with the *literature*. Since the Burdock and Flamm article does not summarize previously published material, it is not a review article despite its title.

#### **Burdock and Flamm (1999) is not formatted as a review article.**

Review articles typically do not include “Methods” and “Results” sections. Rather, they include a brief listing of the studies to be included in the review, often in tabular form, usually giving only brief descriptions (if any) of the design of each study. Once the universe of studies to

be reviewed has been identified, the review article typically addresses similarities and differences in the research findings. It is at this stage that study designs and methodologies may be introduced as possible explanations for seemingly inconsistent findings. Finally, the typical review article concludes with a discussion of the implications of the totality of the research reviewed—what relationships appear to have been consistently demonstrated or consistently refuted, what relationships appear to be poorly understood, and what additional research questions seem most paramount.

Burdock and Flamm (1999), on the other hand, is formatted as what it is—a series of toxicity studies of a single compound, polydextrose, presented one after another. The article contains an introduction followed by a “Methods” section in which each study is described, one at a time, as it would be in the “Methods” section of a stand-alone report. This is followed by a “Results” section in which the findings of each study are described, one at a time, as they would be in a stand-alone report. The “Methods” and “Results” sections of Burdock and Flamm could be cut apart with scissors and reassembled into independent reports of each study, a feat that would never be possible with a true review article. Only in the “Discussion” section does the Burdock and Flamm paper abandon its treatment of each study as an independent piece of research and address itself to the conclusions that can be reached from the totality of research.

**Burdock and Flamm (1999) presents analyses based on examination of raw data rather than of summary reports.**

Not only are review articles normally based on the published literature, they are invariably based on reports of the findings of research rather than on direct inspection of the raw data provided by the research. This is not the case with Burdock and Flamm’s article. It was prepared in the same way as journal articles of individual toxicity studies are prepared, by inspection of the raw data as well as the principal investigators’ study reports. George Burdock, the senior author of the article, and Gary Flamm received and reviewed the raw data. Based on this review, Drs. Burdock and Flamm prepared the descriptions of the results of each study and the conclusions from the research as a whole. In this regard it should be noted that Burdock and Flamm take full responsibility and credit for the results and conclusions by not citing previous researchers. The scientists who actually conducted the toxicity studies are listed alphabetically in the “Acknowledgments” paragraph, where they are credited only with “carrying out the studies herein.” They are not credited with analyzing, reporting, or interpreting the findings of the studies.

**Burdock and Flamm (1999) describes the research methods and results at a level of detail consistent with original publication rather than a review.**

We wish to emphasize, as FDA is of course also aware, that the need for general availability of safety information in GRAS determinations is to assure that “general recognition” of safety by the scientific community is based on sufficient information to allow judgment of the power and validity of the data on which the safety determination is based. Review articles rarely provide sufficient detail to allow readers to evaluate the quality of the studies reviewed. We believe that Burdock and Flamm is critically different in that it does present the toxicity research in sufficient detail to allow readers to evaluate the research, and in that it is based on primary rather than secondary assessment of the research data.

Burdock and Flamm (1999) present a number of safety studies:

- Single dose and LD<sub>50</sub> studies in mice, rats and dogs
- 3-Month gavage study in monkeys with the neutralized form of polydextrose (PDX-N)
- 3-Month feeding study in dogs with the acidic form of polydextrose (PDX-A) at 50% of the diet
- 6-Month feeding study in dogs with PDX-N, followed by PDX-A at 50% of the diet
- 13-Month feeding study in beagle dogs with PDX-A
- 24-Month toxicity study in beagle dogs with PDX-N at 10% and 20% of the diet
- 24-Month toxicity study in beagle dogs with PDX-N at 50% of the diet
- 18-Month carcinogenicity study in mice with PDX-A
- 3-Month dietary study in rats with PDX-A
- 24-Month carcinogenicity study in rats with PDX-A
- Segment I study in rats (fertility and general reproductive performance)
- Segment II study in rats (pregnancy and fetal development)
- Segment III study in rats (study of the action of PD on the perinatal and postnatal development of the rat)
- Three-generation study in rats
- Segment II study in rabbits (pregnancy and fetal development)
- Spot test using *Salmonella typhimurium*
- Quantitative plate assay using *Salmonella typhimurium*
- Host mediated assay with PDX
- Cytogenetic analyses (both *in vivo* and *in vitro* studies)
- Dominant lethal assay in mice

Because the current forms of polydextrose are direct descendents of PDX-A (in that they do not contain added potassium hydroxide or potassium carbonate as does PDX-N), the studies that employed PDX-A were regarded as more important in establishing the GRAS status of the use of current forms of polydextrose in infant formula than the studies that used PDX-N. Nevertheless, the latter studies are also discussed.

The most important studies are the repeat-dose toxicity studies. The first of these is the 3-month feeding study in beagle dogs with PDX-A. For this study, the information provided in the "Methods" section included:

- Number, strain and sex of dogs
- Source (Marshall Farms)
- Age
- Full description of the feed to which PDX was added
- Amount of feed provided at different stages as the dogs grew
- Vitamin and mineral supplementation
- Length and time of day of the feeding period
- Availability of water
- PDX doses
- Frequency of observation
- Frequency of weighing
- Frequency of measurement of water consumption

- Frequency of clinical pathologies
- Times of day of blood draws for serum chemistries
- Tissues taken for examination at necropsy

This information is at least as extensive as is typically found in stand-alone publication of similar toxicity studies. The results reported from this study also match the level of detail that would be expected in a stand-alone publication, including:

- Mortality
- Weight and weight gain
- Stool characteristics
- Water consumption
- Serum calcium concentrations
- Urinary sodium concentrations
- Statement of no differences in hematology; histopathology; or absolute or relative organ weights

Another key study is the 13-month feeding study in beagle dogs with PDX-A. Again, extensive information (requiring more than 12 column-inches) was provided regarding the study design and methods:

- Number, strain and sex of dogs
- Source (Marshall Farms)
- Age
- Full description of the feed to which PDX was added
- Frequency of mixing of PDX into feed
- Length and time of day of the feeding period
- PDX doses in both concentration in the feed and approximate g/kg bw/day
- Frequency of observation
- Frequency of weighing
- Frequency of ophthalmoscopic examination and type of ophthalmoscope used
- Frequency of measurement of ECG, blood pressure, heart rate, respiratory rate, and rectal temperature
- Frequency of measurement of water consumption
- Frequency of clinical pathologies (serum chemistry, hematology, and urinalysis)
- Times of day of blood draws for serum chemistries
- Full list of parameters measured in clinical pathologies
- Full description of the animal sacrifice, necropsy, organs weighed and subjected to histopathology
- Preparation of tissues for histopathology and staining methods used
- Description of the statistical testing

The results of this study were again reported at a level of detail consistent with original publication of toxicity studies, requiring more than 20 column-inches. (In the “Results” section

of Burdock and Flamm (1999), this study is mislabeled as having employed PD-N rather than PD-A.) Findings reported include:

- Mortality
- Water consumption
- Bodyweight gain
- Stool consistency at different times after feeding
- Incidence of diarrhea
- Report of no differences in ECG, systolic blood pressure, vital signs, ocular lesions, or hematology parameters
- Serum calcium levels throughout the study
- BUN levels throughout the study
- Urinary volume, pH, specific gravity, sodium levels, and potassium levels
- Urinary calcium levels throughout the study
- Histopathology findings, especially kidney lesions

Another key long-term study is a 24-month toxicity study in beagle dogs of PDX-N at 10% and 20% of the diet. Although this research employed PDX-N rather than PDX-A, it was regarded as particularly important because of its 2-year duration. The “Methods” section for this study is extensive and includes:

- Number, strain and sex of dogs
- Source (CERM-RIOM, France)
- Age
- Detailed description of the feed and the feeding procedures
- PDX doses as concentration in the feed and approximate g/kg bw/day
- Frequency of observation and weighing
- Frequency of ophthalmic evaluations
- Frequency and descriptions of pulmonary and cardiac auscultation, abdominal palpation, inspection of teeth and skin
- Frequency and descriptions of hematology, clinical chemistry, and urinary, evaluations
- Description of liver analyses, necropsy, tissue sections

The report of the results of this study occupy 2½ pages and include tables of body weights by quarter by sex and group, incidence of elevated plasma calcium values by quarter by sex and group, and renal lesions related to plasma calcium levels. The results reported include:

- Mortality
- Changes in body weights
- Differences in feed consumption
- Differences in observed behavior
- Differences in body fat deposition
- Incidence and severity of of diarrheal episodes
- Incidence of blood in the stools
- Incidence of vomiting

- Incidence of galactorrhea
- Ophthalmologic findings including lesions
- Hematology including differences in hemoglobin, red blood cells, and hematocrit
- Plasma calcium, sodium, urea, and serum triacylglycerol values
- Urinalysis including lowered osmolality
- Absolute and relative organ weights
- Histopathology findings, including extensive discussion of kidney lesions related to plasma calcium levels

The other long-term studies, such as the carcinogenicity and developmental toxicity studies, were described and reported in similar or even greater detail. In all, Burdock and Flamm includes 37 tables of results of the long-term toxicity studies, certainly comparable to the information that is available in many stand-alone publications of toxicity studies.

In summary, Burdock and Flamm (1999), despite its title, is not a review article. It does not meet the definition of a review article as one that summarizes previously prepared study reports. It is formatted as a research report rather than as a review, with sections on “Methods” and “Results,” followed by “Discussion.”

Most important for regarding Burdock and Flamm as original reporting rather than as a review is that it is based on primary analysis and interpretation of the raw data from the toxicity studies rather than being based on examination of reports of the data analysis. And most important for regarding Burdock and Flamm as suitable for providing a basis for determining general recognition of safety—GRAS—is that it provides sufficient detail about the design and results of each toxicity study to allow members of the scientific community to judge the adequacy of the preclinical data (along with other published information regarding kinetics as well as human studies) to establish the safety of polydextrose under its intended conditions of use.

In our opinion, FDA can agree that the data from Burdock and Flamm (1999) are appropriately used as part of the GRAS determination for the use of polydextrose in infant formula without compromising the recognized GRAS requirement that such data must be original information generally available to the scientific community.

The types of preclinical safety studies reported by Burdock and Flamm (1999) include genotoxicity, acute toxicity, subchronic toxicity, chronic toxicity, carcinogenicity and reproductive/developmental toxicity studies. Because of the extensive amount of data generated, the findings from preclinical *in vivo* studies of orally administered polydextrose (acidic and neutral forms) are presented in Table 17. *In vitro* preclinical studies for polydextrose (acidic form) and detailed explanations of the methods and findings of the oral *in vivo* preclinical studies are described in pages 48 through 63. The experimental data obtained from all these preclinical safety studies are critically evaluated to identify any potential hazards from ingesting polydextrose. Because the polydextrose product, Litesse<sup>®</sup> Two produced by Danisco is the acidic form, the final safety assessment is based on studies that administered the acidic form rather than the neutral form. Throughout the rest of this document, the use of terms indicating significant differences from control group (e.g., increase, decrease and reduced) were based on the statistical procedures used.

Table 17. Summary of Experimental Studies in Animals Fed Acidic PDX or Neutral PDX

Species (gender)	Study Type	Strain	n per group	PDX Form	Route	Dose(s) (g/kg)	Duration (days)	Results
Mice (M)	Acute (single dose)	CD-1	10	Acidic	Gavage	6.1, 18.3 or 30.5	7 <sup>c</sup>	Oral LD <sub>50</sub> >30.5 g/kg body weight. Soft stools at highest dose administered
Mice (M)	Acute (single dose)	CD-1	10	Acidic	Gavage	9.5, 28.4 or 47.3	7 <sup>c</sup>	Oral LD <sub>50</sub> >47.3 g/kg body weight Soft stools at highest dose administered
Mice (M/F)	Carcinogenicity (repeat, multi-dose)	CD-1	50	Acidic	Feed	0, 7.5 or 15	528	<u>Unaffected</u> Survival, clinical symptoms, clinical chemistry, hematology, ocular lesions, body weight, organ weights, post-mortem gross examination, tumor incidence <u>Observed Changes</u> Mean liver weight reduced 13% in males fed 7.5 g/kg and unaffected at higher dose. This observation is toxicologically insignificant
Rats (M)	Acute (single dose)	Sprague-Dawley	8	Acidic	Gavage	4.7, 12, 18.9	7 <sup>c</sup>	Oral LD <sub>50</sub> >18.9 g/kg body weight Soft stools at highest dose administered
Rats	Subchronic (repeat, multi-dose)	Charles River CD	13	Acidic	Feed	0, 1, 2 or 10	92	<u>Unaffected</u> Body weights, physical and clinical pathologies, ocular lesions, histopathology
Rat (M/F)	Carcinogenicity (repeat, multi-dose)	Sprague-Dawley	50	Acidic	Feed	0, 5 or 10	704	<u>Unaffected</u> Survival, food intake, growth, ocular lesions, clinical chemistries, hematology (adult rats), organ weights, autopsy and histological abnormalities, tumor incidence <u>Observed Changes</u> Soft and dark feces. Hemoglobin and RBC were increased (M & F weanling at 10 g/kg), WBC decreased (F weanling at 10 g/kg)
Dog	Subchronic (repeat, multi-dose)	Beagle	6 PDX 4 control	Acidic	Feed	0, 21	90	<u>Unaffected</u> Serum calcium, hematology, histopathological abnormalities <u>Observed Changes</u> Loose unforned to partially formed (occasionally watery) stools. Urinary sodium concentration was reduced on treatment days 43 and 71, and unaffected at other time points tested. The observed urinary change is considered toxicologically insignificant

Species (gender)	Study Type	Strain	n per group	PDX Form	Route	Dose(s) (g/kg)	Duration (days)	Results
Dog (M&F)	Chronic (multi-dose)	Beagle	5	Acidic	Feed	0, 7 or 14	407	<p><u>Unaffected</u> mortality, body weight gain, serum chemistry (7 g/kg), urinary chemistry (7 g/kg), hematology, pathological abnormalities</p> <p><u>Observed Changes</u> The 7 g/kg PDX treatment resulted in loose and unformed stools, while 14 g/kg PDX resulted in watery stools. The stool softening effects occurred within hours after dosing and was a transient effect. Water consumption was increased in the 14 g/kg group and unaffected in the lower dose group. Serum and urinary chemistries were intermittently affected in only a few dogs treated with 14 g/kg PDX. The serum and urinary changes are considered toxicologically insignificant.</p>
Rats (M)	Acute (single dose)	Sprague-Dawley	8	Neutral	Gavage	4, 7, 12 or 18.8	7 <sup>c</sup>	<p>Oral LD<sub>50</sub> &gt;18.8 g/kg body weight</p> <p>Soft stools at highest dose administered</p>
Dog (M)	Subchronic (single dose)	Beagle	4	Neutral (days 1-135) Acidic (days 136-195)	Feed	0, 31 (neutral), 21 (acidic)	195	<p><u>Unaffected</u> Body weight gain, serum calcium, serum potassium, BUN, serum glucose, serum LDH, serum CO<sub>2</sub>, urinary potassium, urinary phosphate</p> <p><u>Observed Changes</u> Transient diarrhea (subsided within 24 hrs) was observed in PDX (neutral form) treated dogs, which was absent in dogs treated with PDX (acidic form). Only loose and unformed stools were observed in dogs treated with PDX (acidic form). Serum calcium and potassium were transiently increased, and BUN reduced, in PDX (neutral form) treated dogs and not in dogs treated with PDX (acidic form). Urinary sodium fluctuated less in PDX (neutral form) treated dogs, whereas it was unaffected by PDX (acidic form) compared to control. Urinary calcium increased somewhat (data not quantified) by PDX (acidic form) and unaffected by PDX (neutral form). In two clinically hypercalcemic dogs treated with PDX (neutral form), calcium nephropathy was observed that was attributed to the diarrhetic state induced by neutral PDX. Calcium nephropathy was absent in the other two dogs.</p>

000296

Species (gender)	Study Type	Strain	n per group	PDX Form	Route	Dose(s) (g/kg)	Duration (days)	Results
Dog (M&F)	Chronic (multi-dose)	Beagle	6	Neutral	Feed	0, 2 or 4/8	760	<p><u>Unaffected</u> body weights, food consumption, pulmonary and cardiac auscultation, palpation of abdomen and lymph nodes, teeth, skin, urogenital area, osteotendinous reflexes and ECG recordings, platelet count and white blood cell, fibrinogen partial thromboplastin time, prothrombin time, plasma sodium and triglyceride, urinary glucose, ketone bodies, urobilin, proteins, blood, sediments, liver lipids, organ weights</p> <p><u>Observed Changes</u> Anemia occurred in two dogs treated with 4 g/kg (anemia was absent in the other four dogs) Plasma calcium exceeded 115 mEq/liter in one dog and only after 6 months of treatment with 2 g/kg PDX Plasma calcium was increased in two to four male dogs after 4 months of treatment and in female dogs after 1 month of treatment Plasma urea was normal for the first 18 months of treatment, after which it was increased in dogs treated with 8 g/kg PDX Nephrocalcinosis effect was observed in a few PDX treated dogs but was not considered treatment related, no other pathological findings were found Severe diarrhea occurred during the first week of treatment at both dose levels, and then it was mild to moderate for the remainder of the study</p>
Dog (M&F)	Chronic (single dose)	Beagle	6	Neutral	Feed	0 or 21.5	760	<p><u>Unaffected</u> serum chemistry, hematological parameters, ocular lesions, estrous period (F only).</p> <p><u>Observed Changes</u> Body weight gain was reduced and was associated with varying degrees of anorexia, however, this effect was reversed during the recovery period Diarrhea also occurred in PDX treated dogs that also stopped within three days of stopping PDX treatment</p>
Monkey (M&F)	Subchronic (multi-dose)	Rhesus	4	Neutral	Gavage	0, 1, 2 or 10	90	<p><u>Unaffected</u> clinical observations, ocular lesions, ECG parameters</p> <p><u>Observed Changes</u> Loose stools were consistently observed in the 10 g/kg group, but not in monkeys treated with the lower two doses Serum calcium was reduced on 2 days in 1 to 4 monkeys treated with 2 or 10 g/kg One monkey treated with 10 g/kg PDX had hematuria and trace proteinuria All monkeys in the highest dose group had moderate dilatation of colon and hemosiderin accumulation in colonic mucosa due to loose stools</p>
Source Burdock and Flamm (1999)								

000297

### a. Genotoxicity

#### (1) *In Vitro*

Pfizer, Inc. investigated the genotoxicity of polydextrose using *Salmonella typhimurium* (i.e., spot test and plate assay) and human lymphocytes (cytogenetic analysis) (Burdock and Flamm 1999).

The mutagenic activity of polydextrose (acidic and neutral forms) was tested in *S. typhimurium* (strains TA1535, TA1536, TA1537, TA1538 and TA1978) using the spot test in which colonies are grown in a histidine-deficient environment and wherein revertant colonies appear as a ring surrounding the point of application of polydextrose (Ames 1971). Cells were incubated with polydextrose for 48 hours at 37°C and 18 hours at 25°C. The plate assay (Ames et al. 1973) was used to assess the induction of base-substitution and frame-shift point mutations. Tester strains (TA1535, TA1536, TA1537 or TA1538) were incubated with polydextrose (acid or neutral forms at 10 or 20 mg/plate) for 60 hours at 37°C and the number of revertant colonies recorded. A three-fold induction of revertants over control was considered a positive response. In the spot test, no mutagenic activity was observed in any of the strains tested. In the plate assay, polydextrose did not produce a positive mutagenic response at either 10 or 20 mg/plate. (Burdock and Flamm 1999)

Clastogenic activity (i.e., chromosomal damage) of polydextrose (acidic and neutral forms) was evaluated using human lymphocytes. Polydextrose (500 or 1000 µg/ml) was incubated with lymphocytes for 24 hours, and then harvested, stained and the number of cells with structural aberrations was recorded. Polydextrose did not induce chromosomal aberrations in this *in vitro* assay (Burdock and Flamm 1999).

A host-mediated assay was performed using *S. typhimurium* and CD-1 mice. Mice ( $n=4$  per group) were implanted with *S. typhimurium* by intraperitoneal injection with a 2 ml solution of cells ( $10^7$  cells/ml). At 0, 1 and 2 hours after implantation, polydextrose (acidic and neutral forms) was administered. Thirty minutes after the last dose, 1.5 ml solution of saline-citrate was injected intraperitoneally into each mouse. Mice were then killed by cervical dislocation and fluid was recovered aseptically from the peritoneal cavity. Cells in the collected fluid were then cultured and the mutation frequencies (i.e., the number of revertants) were recorded. Polydextrose failed to induce a significant increase in mutation frequency compared to control. Burdock and Flamm (1999) reported that elevated mutation frequency occurred in the first assay; however, this effect was not reproducible in subsequent assays. Burdock and Flamm considered the initial increase in mutation frequency to be a spurious event.

#### (2) *In Vivo*

The potential for any clastogenic activity on the part of polydextrose (acidic and neutral forms) was investigated *in vivo* using CD-1 mouse bone marrow cells (Burdock and Flamm 1999). Mice ( $n=5$  per group; 30-40 grams body weight) were administered polydextrose (2 g/kg bw) orally and killed at 6, 12, 24, 48 or 72 hours post-treatment. An additional group was included in which mice received polydextrose (neutral form) orally, daily for seven days, and were then killed 24 hours after the last treatment. Three hours prior to sacrifice, each animal was injected intraperitoneally with colchicine (1 mg/kg bw) to inhibit cell division. Bone marrow cells from the femur were collected, stained and examined microscopically for chromosomal

damage. Polydextrose did not induce chromosomal aberrations above internal or historical control values, indicating a lack of clastogenic activity.

### (3) Summary of Genotoxicity

These *in vitro* genotoxicity data indicate that polydextrose (both acidic and neutral forms) is neither mutagenic nor clastogenic. Thus, polydextrose does not damage the genetic component of cells. Based on these results, it is unlikely that polydextrose is carcinogenic. Because it is difficult to extrapolate *in vitro* data to *in vivo* animals and humans, these results are only suggestive; a definitive confirmation of no carcinogenic activity by polydextrose can only be established with *in vivo* carcinogenicity studies. Nevertheless, these *in vitro* studies demonstrate that polydextrose is not directly genotoxic in cultured cells.

These *in vivo* genotoxicity data indicate that polydextrose does not adversely interact with DNA in the intact animal under the conditions tested. This finding is examined more rigorously in traditional toxicity tests.

### b. Acute Toxicity

The acute oral toxicity of polydextrose (acidic form) was investigated in mice, rats and dogs. The strain, gender, dose, route and observation period are presented in Table 18. Polydextrose (acidic form) was investigated in mice, rats and dogs (oral route), while polydextrose neutral form was investigated in rats (oral) and dogs (intravenous injection). The median lethal dose (LD<sub>50</sub>) was calculated after administration of increasing doses of polydextrose and recording mortality over the 7-days post administration. During the observation period, signs of toxicity were also recorded. At study termination, mice and rats were necropsied for gross pathological changes while dogs were remanded to stock.

Table 18. Acute Toxicity of PDX in Mice, Rats and Dogs

	Mice		Rat		Dog	
	PD-A	PD-N	PD-A	PD-N	PD-A	PD-N
Strain	CD-1	CD-1	CD (SD)BR	CD (SD)BR	NS	NS
Gender	M	M	M	M	M/F	F
Number per Group	10	10	8	8	2	2
Aqueous Solution	50%	70%	50%	70%	70%	70%
Doses (g/kg bw)	6.1, 18.3 or 30.5	9.5, 28.4 or 47.3	4.7, 12, 18.9	4.7, 12 or 18.8	20	1.2 or 2
Route	oral <sup>†</sup>	oral <sup>†</sup>	oral <sup>†</sup>	oral <sup>†</sup>	oral <sup>**</sup>	<i>iv</i>
Observation Period (days)	7	7	7	7	7	7
LD <sub>50</sub> (g/kg)	≥30.5	≥47.3	≥18.9	≥18.8	≥20	≥2
PD-A = polydextrose acidic form, PD-N = polydextrose neutral form, <i>iv</i> = intravenous, NS = not specified, <sup>†</sup> Oral route was gavage, <sup>**</sup> Oral route was gavage and as a capsule containing polydextrose powder, Number of mice, rats and dogs per group was not specified Source: Burdock and Flamm (1999)						

### (1) Mice

Two LD<sub>50</sub> studies of polydextrose (acidic form) were performed in mice. In the first study, male mice (n=10 per group; bw between 23 and 28 grams) were fasted over night and then gavaged with increasing doses (i.e., 6.1, 18.3 or 30.5 g/kg bw) of polydextrose (acidic form). No mortality occurred during the 7-day observation period after polydextrose administration, indicating that the oral LD<sub>50</sub> exceeds 30.5 g/kg bw in mice. At the higher two doses (i.e., 18.3 and 30.5 g/kg bw) mice exhibited face rubbing immediately after polydextrose administration. Diarrhea occurred at all three doses but subsided after 24 hours. No other symptoms occurred. Body weight gain was unaffected and internal gross pathologies were absent at necropsy. (Burdock and Flamm 1999)

In the second study, male mice (n=10 per group; bw between 18 and 22 grams) were fasted over night and then gavaged with increasing doses (i.e., 9.46, 28.38 or 47.3 g/kg bw) of polydextrose (acidic form). One mouse died 24 hours after administering 47.3 g/kg bw polydextrose (acidic form). All 29 remaining mice survived the 7-day observation period. Diarrhea occurred at all dose levels, but not in all mice at each dose level. At the lowest dose of 9.46 g/kg bw, two mice had diarrhea, while six mice administered 28.38 g/kg bw had diarrhea. At the highest dose of 47.3 g/kg bw, all ten mice had diarrhea. Mice that were administered 28.38 or 47.3 g/kg bw exhibited occasional blanching, depression and occasional retching. At the highest dose (i.e., 47.2 g/kg bw), but not at the lower two doses, mice exhibited signs of toxicity, which were ataxia, decreased respiration, occasional tendency for prostration, occasional tremors and weakness. Symptoms of toxicity began within 15 seconds of administration and subsided within 24 hours. Weight gain was unaffected and internal gross pathologies were absent at necropsy. (Burdock and Flamm 1999)

These studies demonstrate that the oral LD<sub>50</sub> in mice exceeds 47.3 g/kg bw, the highest dose tested, polydextrose (acidic form).

### (2) Rats

Two LD<sub>50</sub> studies were performed in rats administered either polydextrose neutral or acidic forms. In the first study, male rats (n=8 per group; 51-63 gram bw) were fasted over night and then gavaged with increasing doses (i.e., 4.7, 12 or 18.8 g/kg bw) of polydextrose (neutral form). Mortality was absent in the three groups of rats administered polydextrose, indicating that the oral LD<sub>50</sub> exceeds 18.8 g/kg bw in rats. The only observable symptom was diarrhea that occurred at all dose levels. At the lowest dose of 4.7 g/kg bw, four rats exhibited diarrhea, while seven rats had diarrhea at the middle dose of 12 g/kg bw. At the highest dose of 18.8 g/kg bw, all eight rats had diarrhea. All cases of diarrhea had resolved within 24 hours of polydextrose administration. Body weight gain was unaffected at all dose levels and internal gross pathologies were absent at necropsy (Burdock and Flamm 1999).

In the second LD<sub>50</sub> study, male rats (n=8 per group; 55-70 grams bw) were fasted over night and then gavaged with increasing doses (i.e., 4.7, 12.0 or 18.9 g/kg bw) polydextrose (acidic form). Mortality was absent at all dose levels of polydextrose, which indicates that the oral LD<sub>50</sub> in rats exceeds 18.9 g/kg bw. The only observable symptom was diarrhea that occurred at all three doses levels. At the lowest dose of 4.7 g/kg bw, diarrhea occurred in four of the eight rats, while diarrhea occurred in all rats of the higher two dose groups (12.0 and 18.9 g/kg bw). It was noted that diarrhea was more watery at the higher two doses than at the lowest dose.

Consistent with the previous LD<sub>50</sub> studies, the diarrhea lasted for 24 hours, and then subsided. Weight gain was unaffected and internal gross pathologies were absent in all three groups. (Burdock and Flamm 1999)

The data indicate that the oral LD<sub>50</sub> in rats for both forms of polydextrose (*i.e.*, acidic and neutral) exceeds 18.9 g/kg bw.

### (3) Dogs

Intravenous and oral LD<sub>50</sub> studies were conducted in beagle dogs. In the intravenous study of polydextrose (neutral form), two female dogs (11.0 and 8.9 kg bw) were fasted overnight, and then, one dog was administered 1.2 g/kg bw polydextrose (neutral form), while the other dog was administered 2 g/kg bw. Both dogs survived the 7-day post administration period. Signs of toxicity were absent. Diarrhea was also absent in both dogs (Burdock and Flamm 1999).

In the oral study of polydextrose (acidic form), one male and one female dog (12.7 and 10.5 kg bw, respectively) were fasted over night, and then intubated with 20 g/kg bw polydextrose. Mortality did not occur in either of the two dogs during the post 7-day observation period. The investigators noted that severe signs of toxicity were also absent. In the female dog, emesis occurred one hour after dosing, as well as polydipsia after consuming water 1.5 hours after dosing. Soft stools were observed in the female dog 24 and 72 hours after polydextrose administration. In the male dog, diarrhea was observed, as well as polydipsia, 1.5 hours after dosing. The diarrhea subsided within 24 hours of polydextrose administration. Emesis also occurred during the evening after polydextrose administration in the male dog. Food consumption and body weight gain were normal in both dogs. (Burdock and Flamm 1999)

Although the number of dogs in each group is low, these data indicate that the intravenous and oral LD<sub>50</sub> exceed 2 g/kg bw and 20 g/kg bw, respectively, in beagle dogs.

### (4) Summary of Acute Toxicity

The data indicate that under acute oral exposure conditions, exceedingly high doses of polydextrose are required to produce overt signs of toxicity and mortality in mice rats and dogs. Thus, polydextrose presents very low toxicity when administered orally. The only consistent observable effect in all three species at these very high doses was diarrhea, which lasted for less than 24 hours post-administration. The incidence and severity of diarrhea were dose-dependent. Because acute exposure conditions are not representative of the proposed exposure conditions in infant formula, these data can only be considered as corroborative of an overall low order of oral toxicity of polydextrose.

### c. Subchronic Toxicity

Pfizer, Inc. conducted subchronic (*i.e.*, 30 to 90 days in duration) toxicity studies in which polydextrose (acidic form) was administered to rats and dogs, while polydextrose (neutral form) was administered to monkeys. One subchronic study was performed with dogs in which polydextrose (neutral form) was administered for 135 days followed by administration of polydextrose (acidic form) for a further 57 days. The studies of polydextrose (acidic form) are presented on page 53, while the study of polydextrose (neutral form) is presented on page 53. Because the polydextrose products produced by Danisco and proposed for use in infant formula

are polydextrose (acidic form), the safety evaluation is based on the results of studies of polydextrose (acidic form).

(1) Polydextrose (acidic form)

(a) Rat

The subchronic oral toxicity of polydextrose (acidic form) was investigated in male and female rats (Charles River CD). Rats were fed either a basal diet or a diet containing polydextrose (1, 2 or 10 g/kg bw) for 92 days (Burdock and Flamm 1999). Body weights and food consumption were recorded weekly and the amount of polydextrose administered was adjusted accordingly. Hematology [erythrocyte (RBC), leukocyte (WBC) and WBC differential counts, hemoglobin concentration, hematocrit and whole blood clotting time] and urinalysis examination were performed prior to treatment and on treatment days 29, 57 and 85. Clinical chemistry [fasting blood sugar, blood urea nitrogen (BUN), serum glutamic-pyruvic (SGPT) and serum glutamic-oxaloacetic transaminase (SGOT), serum alkaline phosphatase, total bilirubin, serum lactic dehydrogenase (LDH), serum creatinine phosphokinase (CPK), serum sodium ( $\text{Na}^+$ ), serum calcium ( $\text{Ca}^+$ ), serum creatinine and uric acid levels] was examined in two rats per sex per dose on treatment days 36 and 64, as well as in six rats per sex per dose on treatment day 92. Slit-lamp ophthalmoscopic examinations were performed on each animal on days 29, 57 and 85. When the study was terminated, organ weights [heart, lung, liver, kidney, pancreas, spleen, adrenal, thyroid, brain, hypophysis, testis, epididymis, uterus and ovary], gross pathology and histopathology (lung, liver and kidney) were recorded (Burdock and Flamm 1999).

Body weights were unaffected by polydextrose treatment at any of the doses administered. Physical, clinical pathology, ophthalmology, organ weights, gross pathology and histopathology were also unaffected by polydextrose (acidic form) treatment over the 92 day treatment period. The effect of polydextrose on excreted fecal matter was not reported (Burdock and Flamm 1999). The no observed adverse effect level (NOAEL) in this study was 10 g/kg bw/day, the highest dose tested.

(b) Dog

Beagle dogs (10 months old; 10 kg bw) were fed either a basal diet (n=4) or a diet supplemented with polydextrose (acidic form) (n=6) at a level of 50% of the dry weight of the entire ration (100 g canned dog food, 150 g Purina Dog Chow<sup>®</sup>, 17.5 g anhydrous calcium phosphate dibasic and 1 ml Vi-Daylin vitamin drops) for 97 days (approximately 3 months) (Burdock and Flamm 1999). On treatment day 35, the amount of food consumed was increased to 120 g canned and 185 g Purina Dog Chow<sup>®</sup> (i.e., the amount of polydextrose added to the diet was 210 g or 21 g/kg bw). All dogs were observed twice daily. Body weights were recorded daily for the first 3 days, and then weekly thereafter. Water consumption was recorded on days 1-3 and 6-9. Clinical pathology profiles for serum chemistry (sodium, potassium, calcium, glucose, blood urea nitrogen-BUN, uric acid, creatinine, total bilirubin, alkaline phosphatase-ALP, lactic dehydrogenase-LDH, aspartate transaminase-SGOT, chloride,  $\text{CO}_2$ , total protein, albumin, inorganic phosphate, pH and  $\text{pCO}_2$ ) hematology (white blood cell-WBC, red blood cell-RBC, hemoglobin, hematocrit and mean corpuscular volume-MCV) and urinalysis (volume, pH, sodium, potassium, calcium and phosphate) were performed twice prior to treatment and then on treatment days 1, 2, 3, 15, 30, 43, 57, 71, 85 and 97. Urine samples were collected by catheterization at 2 hour intervals between 5 am and 5 pm. Animals were subjected to necropsy

at study termination and tissues collected for macroscopic (liver, kidney, testis) and microscopic (brain, cervical spinal cord, hypophysis, eye, thyroid, thymus, heart, lung, spleen, pancreas, kidney, adrenal, esophagus, stomach, duodenum, jejunum, ileum, colon, mesenteric node, testes, epididymides, prostate gland, urinary bladder, mandibular salivary gland, mammary gland with skin, gall bladder and any other grossly altered tissue) examination (Burdock and Flamm 1999).

All dogs survived the 97-day treatment period. All dogs treated with polydextrose lost weight (0.6-2.2 kg), while 75% of the control group also lost weight (0.5-1.8 kg). Polydextrose (acidic form) treatment resulted in loose unformed to partially formed and occasionally watery stools 3-6 hours after feeding for the duration of the study. Treated dogs drank 2-2.5 times more water than the controls. Except for stool consistency, all treated dogs appeared to be clinically unaffected by polydextrose feeding. Serum calcium concentration was unaffected by polydextrose treatment. On treatment days 43 and 71, mean urinary sodium concentration appeared to be reduced (62% and 54%, respectively), while it was normal at the other time points tested. In the control group, mean urinary calcium concentration declined over the 97-study period (i.e.,  $2.21 \pm 0.71$  mg/l on day 0 and  $0.67 \pm 0.47$  mg/l on day 97). In the polydextrose (acidic form) group, urinary calcium was initially  $1.28 \pm 1.11$  mg/l on day 0 and ranged between  $1.5 \pm 1.22$  and  $2.72 \pm 1.14$  mg/l throughout the treatment period. Because mean urinary calcium concentration was not appreciably affected compared to control (2.21 mg/l control versus 2.72 mg/l polydextrose acidic form) and the high variability in this parameter, urinary calcium was considered to be unaffected by polydextrose (acidic form). Hematological parameters were within normal values. Histopathological parameters and absolute or relative weights of liver, kidney or testes were unaffected (Burdock and Flamm 1999).

## (2) Polydextrose (neutral form)

### (a) Monkey

A three-month subchronic toxicity study of polydextrose (neutral form) was conducted in male and female rhesus monkeys (Burdock and Flamm 1999). Monkeys ( $n=2$  per group; based on result section may be  $n=4$ ) were gavaged daily with polydextrose (0, 1, 2 or 10 g/kg bw) for 91 days. Body weights were recorded weekly and individual doses were adjusted accordingly. The 10 g/kg bw dose was administered as 5 g/kg bw twice daily and delivered in a 50% solution. The 1 and 2 g/kg bw doses were administered in a 70% solution. Hematology, serum chemistry and urinalysis were performed on each monkey twice prior to the start of treatment and on days 29, 57 and 85. Ophthalmoscopic examinations and electrocardiographic tracings were made twice prior to treatment and on days 29, 57 and 85. All animals were killed and necropsied 24 to 28 hours after the last dose. Weights of the following organs were recorded: heart, lung, liver, kidney, pancreas, spleen, adrenal, thyroid, brain, hypophysis, testis, epididymis and prostate. Microscopic examination was performed on the brain, spinal cord, hypophysis, eye, sternum, aorta, liver, spleen, pancreas, kidney, adrenal, esophagus, stomach, duodenum, jejunum, ileum, colon, mesenteric node, reproductive tract, urinary bladder, femoral bone marrow, sciatic nerve, mandibular gland, mammary gland with skin, gall bladder and any other organ grossly altered.

There were no fatalities in the control group or any of the test groups during the 91-day treatment study. One female and two males in the control group gained body weight (0.2 to 0.4 kg), while the remaining female maintained body weight. All monkeys administered polydextrose (neutral form) also gained weight (0.3 to 0.9 kg). Unformed stools did not occur in the control group and rarely occurred in the 1 and 2 g/kg bw groups. Monkeys gavaged with 10

g/kg bw exhibited diarrhea throughout the treatment period, which did not occur at the lower doses (i.e., 1 and 2 g/kg bw/day). Clinical signs of treatment-related effects were absent. One four monkeys at the 10 g/kg bw dose on day 57 and four on day 85 showed decreases (11% and 16%, respectively) in serum calcium concentration. One of four animals at the 2 g/kg bw dose had decreased calcium concentration on days 29 (34%) and 57 (43%). The investigators concluded that because physical signs suggestive of decreased serum calcium were not observed and no histopathologic lesions were evident, the decreased serum calcium was not considered biologically significant.

One monkey treated with 10 g/kg bw polydextrose (neutral form) showed hematuria and trace proteinuria on days 29 and 85. Hematuria and proteinuria were absent in the other three monkeys administered 10 g/kg bw. Ophthalmoscopic, physical and electrocardiographic findings were not remarkable. Post mortem, all animals treated with 10 g/kg bw had moderate dilatation of the colon and focal areas of accumulation of macrophages containing hemosiderin in the colonic mucosa. The accumulation of hemosiderin was considered by the investigators to be a reflection of prolonged hyperemia of the colonic mucosa in response to the protracted diarrhea. The investigators reported no other treatment-related findings.

#### (b) Dog

Male beagle dogs (n=4 per group) were fed either a basal diet or a basal diet supplemented with polydextrose (neutral form) for 6 months. The dose of polydextrose administered was 50% of the dry weight of the entire ration. Beginning on treatment day 1, dogs were fed 26 g/kg bw polydextrose (neutral form). On treatment day 35, dietary supplements were included in the diet that contained calcium (5.3 g). Because the amount of food administered was increased on treatment day 80, the dose of polydextrose (neutral form) was increased to 31 g/kg bw. Then, on experimental day 136, 21 g/kg bw polydextrose (acidic form) was substituted for polydextrose (neutral form) and continued for the duration of the study (i.e., days 136-192). Dogs were observed twice daily, except for weekends and holidays. Body weights were recorded daily until treatment day 126, after which they were recorded weekly. Water consumption was determined on treatment days 9, 13-17 and 174-177. Clinical pathology (i.e., serum chemistry, hematology and urinalysis) samples were obtained at 7:00 am, 11:00 am and 3:00 pm for a total of 33 days. After termination of the treatment period, necropsies were performed and macroscopic and microscopic examinations were performed (Burdock and Flamm 1999).

All dogs survived the six-month treatment period with polydextrose (neutral and acidic forms). All dogs in the control group and one dog in the polydextrose-treated group lost between 0.5 and 1.9 kg body weight during the study period, while the remaining dogs treated with polydextrose (neutral and acidic forms) gained 0.2 to 1.0 kg body weight. Stools of dogs in the control group appeared normal, whereas dogs treated with polydextrose (neutral form) resulted in watery diarrhea. The diarrhea occurred two to three hours after feeding, and then stools returned to normal by the following morning. Substitution with polydextrose (acidic form) resulted in stools that were less watery and thicker, although still with an unformed consistency. Polydextrose-treated dogs drank two to three times more water than the control group (Burdock and Flamm 1999).

Hematological parameters were unaffected by polydextrose treatment. Burdock and Flamm reported that clinical chemistry exhibited periodicity in response to treatment and reaction to the two forms of polydextrose administered. In the control group, serum calcium

concentration showed normal fluctuations and never exceeded 12 mg/dl. In the polydextrose (neutral form) treated group, serum calcium transiently increased (0.5-1.7 mg/liter) post-prandially, peaking after two hours and returning to baseline after 24 hours. In one dog that was fed polydextrose (neutral form), serum calcium concentration exceeded 12 mg/dl on treatment day 71, but not at the other time points. Serum protein concentration peaked immediately post-prandially. Serum protein concentration in the control group showed small variations with no discernable pattern. After the diet was changed from polydextrose (neutral form) to polydextrose (acidic form), the post-prandial peaks reduced and daily fluctuations became similar to the control group. A similar pattern in polydextrose (neutral form) treated dogs was observed for serum potassium concentration. Serum potassium transiently increased post-prandially, exceeding 6 mg/dl (peak value). Switching the diet to polydextrose (acidic form) resulted in serum potassium fluctuations that resembled the control group. Serum sodium concentration was not consistently affected by polydextrose treatments; thus, no discernible treatment related effect on serum sodium was apparent in dog. Blood urea nitrogen (BUN) was reduced in polydextrose (neutral form) treated dogs at the 11:00 am and 3:00 pm sampling times (never at the earlier 7:00 am sampling time). BUN was unaffected in dogs treated with polydextrose (acidic form). Serum glucose, LDH and CO<sub>2</sub> were not consistently affected by polydextrose treatment.

Burdock and Flamm concluded that the observed changes in serum and urinary calcium, sodium and/or potassium in dogs treated with polydextrose (neutral form) are unlikely to be direct treatment-related results, but rather, are more likely to be indirect results secondary to the diarrhetic effect observed at this high dose in dogs (Burdock and Flamm 1999).

In the control group, urinary sodium concentration exhibited daily transient increases between 9:00 am and 1:00 pm (up to 13.3 meq/four-hour urine sample). Dogs fed polydextrose (neutral form) resulted in smaller peaks that never exceeded 6 meq; whereas fluctuation in urinary sodium concentration of polydextrose (acidic form) treated dogs resembled the pattern of the control group. Urinary calcium concentration was unaffected by polydextrose (neutral form) treatment; while treatment with polydextrose (acidic form) inconsistently increased urinary calcium. In the control group, urinary calcium ranged between 1 and 2 meq/workup, while it was between 1 and 3 meq/workup in the polydextrose (acidic form). The day-to-day variation in urinary calcium concentration of polydextrose (acidic form) was so great that the significance of the increase is unclear. Urinary potassium and phosphate concentrations varied widely without any discernible pattern. Hematological parameters were unaffected by polydextrose (neutral or acidic forms) treatment. The only histopathological lesion observed was calcium nephropathy in two polydextrose-treated dogs that was attributed to the administration of polydextrose (neutral form), but not in the other two polydextrose-treated dogs. Gross pathological and histopathological effects were absent in the other organs and tissues examined of polydextrose treated dogs (Burdock and Flamm 1999).

### (3) Summary of Subchronic Toxicity

Treatment of rats with polydextrose (acidic form), up to 10 g/kg bw for 3 months did not elicit an adverse response. In dogs, oral administration of 21 g/kg bw/day of polydextrose (acidic form) for 97 days had no appreciable or consistent effect on any parameter measured. The only observable effect was loose unformed to partially formed and occasionally watery stools in dogs administered 21 g/kg bw/day polydextrose (acidic form); however, the loose stools did not appear to result in any nutritional deficit or untoward effect and it is important to note that this

laxation effect cannot be regarded as diarrhea. These data indicate that polydextrose (acidic form) did not elicit any toxicological effect in rats or dogs at the doses tested.

Polydextrose (neutral form) administered to monkeys at 10 g/kg bw did not produce a consistent, biologically significant effect in any parameter measured, except diarrhea. Administration of polydextrose (neutral form) to dogs at 31 g/kg bw/day also elicited no consistent, biologically significant effect other than diarrhea. The diarrhea produced in dogs fed polydextrose (neutral form) was more severe than in dogs fed polydextrose (acidic forms). Again, it is appropriate to note that Litesse® Two is a polydextrose (acid form) product, and thus the relevance of the findings regarding polydextrose (neutral form) that differ from those found for acidic form is questionable.

#### **d. Chronic Toxicity**

Chronic toxicity studies described in the following sections, one using polydextrose (acidic form) and two with polydextrose (neutral form), were performed by Pfizer, Inc. and reported by Burdock and Flamm (1999). Both studies were performed with beagle dogs. Chronic studies are particularly important in identifying potential adverse effects on neurological and reproductive organs.

##### **(1) Polydextrose (acidic form)**

A 13-month feeding study was performed in beagle dogs (9-months old) with polydextrose (acidic form). Male and female dogs (n=5 per group) were fed either a basal diet or a basal diet supplemented with polydextrose (16.7% or 33% of the diet; 7 or 14 g/kg bw/day). Dogs were observed twice daily and body weights were recorded weekly. Ophthalmoscopic examinations were performed prior to starting the treatment, after 6 months and 12 months of treatment and finally at study termination. Electrocardiogram (ECG), blood pressure and vital signs (heart rate, respiratory rate and rectal temperature) were recorded prior to starting the treatment and on treatment days 93, 176, 267, 358 and 410. Water consumption was recorded prior to starting the treatment, daily during the first week and at 3-month intervals. Clinical pathology profiles and serum chemistry analysis were performed prior to starting the study and on treatment days 2 (urinalysis only), 15, 29, 58, 85, 113, 141, 169, 204, 232, 260, 288, 330, 351, 379 and 407. Serum chemistry parameters measured were sodium, potassium, calcium, glucose, blood urea nitrogen, uric acid, creatinine, total bilirubin, alkaline phosphatase, lactic dehydrogenase, aspartate and alanine transaminases (SGOT and SPGT), total protein, albumin, globulin and albumin/globulin ratio. Hematology parameters included white blood cell (WBC), red blood cell (RBC), hemoglobin concentration, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). White blood cell differential counts were performed on treatment days 15, 29, 85, 169, 204, 232, 260, 288, 330, 351, 379 and 407. Urinalyses included volume, pH, specific gravity, sodium, potassium and calcium. After 416 days of feeding, the study was terminated and the weights of kidney, liver and testicles were recorded after necropsy. Microscopic pathological examinations were performed on tissue samples from kidney, liver, heart (left auricle and ventricle), aorta, lung (right and left lobes), spleen, thyroid, adrenal, pituitary, gall bladder, thymus, mesenteric lymph node, pancreas, mandibular salivary gland, urinary bladder, mammary gland with skin, esophagus, stomach, small and large intestine, testis, epididymis, prostate gland, ovary, uterus, brain, spinal cord, eye, sternum and bone (femur) with bone marrow.

No fatalities occurred in either test group or control group. Body weight gain was unaffected by polydextrose treatment. Stools in the control group were of normal formed consistency and rarely observed to be soft. In the lower dose group (7 g/kg bw/day), stools were loose and unformed 3-6 hours after feeding. Occasionally, stools were watery. In the high dose group (14 g/kg bw/day), stools were soft, unformed and occasionally watery within three hours of feeding; stools were always more watery at six hours after feeding. Twenty-four hours after feeding, stools were no longer watery, but remained unformed. Blood-tinted stools were observed in several polydextrose-treated dogs at both dose levels; however, this occurrence was described as infrequent and indicative of irritation to the gastrointestinal tract. Dogs fed 14 g/kg bw/day polydextrose drank 1.5 times more water compared to the control group. Water consumption of dogs fed 7 g/kg bw/day was normal.

ECG tracings, indirect systolic blood pressure recordings and vital signs were normal. Ocular lesions were absent in polydextrose-treated dogs. Serum calcium concentrations of control and low dose (7 g/kg bw/day) groups were normal and never exceeded 12 mg/dl. One female and four male dogs fed 14 g/kg bw/day polydextrose experienced occasional (5 days or less) high (>12 mg/dl) serum calcium concentrations; however, these high values never occurred on consecutive days and did not exceed 14 mg/dl. Serum calcium concentrations in the remaining dogs in the high dose group was normal.

Hematology parameters were unaffected by polydextrose (acidic form) treatment. Although one dog in the high-dose group (14 g/kg bw/day) had a BUN value of 50 mg/dl on two occasions (treatment days 379 and 407), BUN was regarded as unaffected by polydextrose (acidic form) treatment. Urinary volume, pH, sodium concentration and potassium concentration were also unaffected by polydextrose treatment. With the exception of two male dogs in the high-dose group, urine with specific gravity less than 1.02 was rarely excreted. The mean urinary calcium concentration in polydextrose-treated female dogs at both dose levels was consistently greater than in the control group; however, the extent of the increase was not reported. Burdock and Flamm (1999) also reported that increased urinary calcium excretion coincided with increased plasma calcium concentration in one female dog. Beginning on treatment day 288 and throughout the rest of the treatment period, urinary calcium concentration gradually increased in one male dog treated with 14 g/kg bw/day polydextrose, but the extent of the increase was not reported. In a second dog treated with the highest dose, urinary calcium concentration increased from treatment day 2 until the end of the study, while urinary calcium concentration was unaffected in the other dogs in this same group. In male dogs fed 7 g/kg bw/day polydextrose (acidic form), there was no difference in urinary calcium concentration levels compared to control.

The two high-dose (14 g/kg bw/day) male dogs that had elevated serum calcium levels and low urinary specific gravity also exhibited macroscopic focal areas of pale discoloration of the kidney cortex with occasional linear white streaks running through the cortex and medulla. Microscopically, this lesion consisted of segmental areas of tubular epithelium; in some regions, the epithelium was absent. There was extensive interstitial fibrosis with focal areas of lymphocytic infiltration. Calcium nephropathy was diagnosed in both dogs. One female dog administered 7 g/kg bw/day polydextrose had a small focal area of chronic interstitial nephritis that was considered to be unrelated to polydextrose treatment. Kidneys from all other males and females appeared normal by macroscopic and microscopic examination.

Thyroid follicular cell hyperplasia was observed in all groups (control, low and high dose) and was considered unrelated to polydextrose treatment. Chronic lymphocytic prostatitis was also observed in several males from all groups and was determined to be a result of repeated catheterization. One male fed 14 g/kg bw/day and four males fed 7 g/kg bw/day had multiple epithelial cysts in the epididymal tubules, which Burdock and Flamm (1999) also attributed to multiple catheterization procedures.

In summary, feeding 14 g/kg bw/day polydextrose (acidic form) for 13 months produced daily episodes of unformed and/or watery stools in all five dogs. In two of the five dogs in the high-dose group, hypercalcemia and calcium nephropathy developed. The investigators reported that increased intestinal absorption of calcium probably contributed to the observed hypercalcemia. The investigators reported that the diarrhea produced fluctuating intravascular fluid volume by wasting water and electrolytes particularly sodium. The resulting imbalance in extracellular fluid volume stimulated renal tubular reabsorption of sodium, as well as calcium and eventually produced calcium nephropathy in two of the five dogs administered the high dose of 14 g/kg bw/day polydextrose (acidic form).

In dogs fed the lower dose of 7 g/kg bw/day polydextrose (acidic form), stools were only occasionally watery, while serum calcium and urinary sodium concentrations were comparable to the control group. Calcium nephropathy was absent in all dogs fed 7 g/kg bw/day for 13 months.

## (2) Polydextrose (neutral form)

A two-year toxicity study was performed in beagle dogs (11-14 months of age) administered polydextrose (neutral form) (Burdock and Flamm 1999). Male and female dogs (n=6 per group) were fed either a basal diet or a basal diet supplemented with polydextrose (10% or 20% of the diet; 4 or 8 g/kg bw/day) for 24 months. Clinical symptoms were recorded daily and body weights recorded weekly. Ophthalmic examinations were performed after 0, 6, 12, 18 and 24 months of treatment. ECG readings were taken at the study's termination. Hematology parameters (hemoglobin, RBC, hematocrit, platelet count, WBC, differential count, plasma fibrinogen, partial thromboplastin time and prothrombin time) were measured prior to starting the treatment and then at 1, 3, 4, 6, 18 and 24 months of treatment. Bone marrow smears (femur) were obtained after 24 months of treatment. Clinical chemistries (sodium, potassium, chloride, calcium, cholesterol, triglycerides, glucose, urea, SGOT, SGPT, alkaline phosphatase, protein, bilirubin, ?-glutamyltransferase (?GT), total protein and albumin at the time of study termination) were measured at 0, 3, 4, 6, 12, 18 and 24 months of treatment. Urinalyses included osmolality, glucose, ketone bodies, urobilin, proteins, blood and microscopy of sediment that were measured after 24 months of treatment. Triglycerides, cholesterol and liver phospholipids were also measured after 24 months of treatment. Plasma sodium, potassium, chloride, proteins and calcium were analyzed after 1 month of treatment. Post-mortem gross pathological examinations included body weight, organ weights of the heart, spleen, liver, kidney, testis and adrenals. Microscopic pathological examinations were performed on tissue samples of the brain, spinal cord, peripheral nerve and surrounding muscle, eye, heart, artery, vein, cervical lymph node, mesenteric lymph node, thymus, spleen, bone, bone marrow, cartilage, skin, salivary gland, tongue, esophagus, stomach, duodenum, ileum, colon, pancreas, liver, gall bladder, pituitary, thyroid, parathyroid, adrenal, larynx, trachea, principal bronchi, lung, kidney, urethra, urinary bladder, urethra, ovary, fallopian tube, uterus, vagina, mammary gland, testis, epididymis, prostate, and any grossly observed tumor or lesion found in tissues not listed above.

There were no fatalities in either test group or in the control group. Mean body weights, food consumption, and behavior were unaffected by polydextrose (neutral form) treatment. All dogs fed polydextrose at either dose level had diarrhea. The severity of diarrhea reported was marginally higher in dogs fed 8 g/kg bw/day polydextrose than in those fed 4 g/kg bw/day. The incidence of severe diarrhea was greatest during the first 2 weeks of treatment. For the remainder of the study, only mild to moderate diarrhea occurred at both dose levels. Diarrhea was absent in the control group. In two male dogs fed 8 g/kg bw/day polydextrose, fecal blood was "marked" that Burdock and Flamm (1999) attributed to irritation resulting from the diarrhea. The appearance of fecal blood for the remaining polydextrose-treated dogs (3 dogs fed 8 g/kg bw/day and all dogs fed 4 g/kg bw/day) was comparable to the control group. Vomiting was unproblematic in control and polydextrose-treated groups.

At 0, 12 and 24 months of treatment, pulmonary and cardiac auscultation; palpation of abdomen and lymph nodes; inspection of teeth, skin and urogenital area; testing of osteotendinous reflexes; and ECG recordings were unaffected by polydextrose treatment. Galactorrhea was observed in two female dogs treated with 5 g/kg bw/day polydextrose, but was reported to be unrelated to the polydextrose treatment as galactorrhea occurs regularly in female dogs, especially following the estrous period, and is occasionally associated with "pseudo-pregnancy." Ophthalmologic examinations revealed post-embryonic, traumatic and spontaneous age-related lesions. Lesions of the eye were reported to be unrelated to treatment. Platelet counts, WBC, fibrinogen partial thromboplastin time and prothrombin time were unaffected by polydextrose treatment.

In male dogs treated with 8 g/kg bw/day polydextrose, a progressive fall in blood hemoglobin, RBC and hematocrit was observed in 75% of the animals by the 24th month of treatment. In females at this same dose, 25% showed progressive anemia. Anemia did not develop in the other treatment groups. The investigators attributed the anemic state to the diarrhea produced by polydextrose (neutral form). Because intestinal bleeding was insufficient to explain the anemic state, the investigators proposed that the underlying cause of the anemia was malabsorption.

The frequency of plasma calcium values that exceeded 115 mEq/liter was increased in polydextrose-treated groups only. After 24 months of treatment with 4 g/kg bw/day polydextrose, elevated plasma calcium was observed in one male. At this same dose, elevated plasma calcium concentrations were observed in one or two female dogs beginning at 6 months and persisted to the end of treatment. At the higher dose of 8 g/kg bw/day polydextrose (neutral form), plasma calcium concentrations were elevated in two to four male dogs per time point at all the time points measured beginning at 4 months of treatment. In female dogs treated with the high dose of polydextrose, the frequency of elevated plasma calcium concentration was 1, 2 and 4 dogs at the 12-, 18- and 24-month time points, respectively. The frequency of elevated plasma calcium concentration was significant in male dogs treated with 8 g/kg bw/day throughout the study period, and in male and female dogs treated with 4 g/kg bw/day polydextrose at the 24-month time-period only. The investigators reported that the increased plasma calcium concentration was due to increased intestinal calcium absorption. Plasma sodium and triglyceride concentrations were unaffected by polydextrose treatment.

Plasma urea concentration was unaffected until month 18 of treatment. In dogs treated with 8 g/kg bw/day polydextrose, urea was increased in both males (45-233 mg/100 ml) and

females (43-109 mg/ml) at the 24-month treatment period. After 24 months of treatment with polydextrose, urinary glucose, ketone bodies, urobilin, proteins, blood and sediments were unaffected in male and female dogs. Reduced osmolality was observed in male dogs treated with 4 g/kg bw/day polydextrose and female dogs treated with 4 or 8 g/kg bw/day polydextrose; however, it is not clear whether this effect was treatment related. Liver lipids (triglycerides, cholesterol and lipid phosphorus) were also unaffected.

Post-mortem analysis revealed organ weights to be unaffected by polydextrose treatment. Nephrocalcinosis was observed in male and female dogs treated with 4 or 8 g/kg bw/day polydextrose and appeared to be dose-related. The investigators postulated that the observed nephrocalcinosis was secondary to the diarrhea produced. Other pathological findings (renal chronic inflammation, papillary calcification and parasitic lesions of the lung) were determined to be unrelated to treatment.

A second two-year toxicity study was performed in beagle dogs treated with polydextrose (neutral form) for 24 months (Burdock and Flamm 1999). Male and female dogs (n=6 per group; 14-15 months old) were fed either a basal diet (250 g) or the basal diet supplemented with polydextrose (neutral form) (50% of the diet; 21.5 g/kg bw/day) for 18 months; then all dogs were fed the basal diet for an additional 6 months (recovery period). Clinical symptoms and body weights were recorded daily and weekly, respectively. An electrocardiogram was recorded prior to treatment and then after 12, 18 and 24 months of treatment. Ophthalmic examinations were performed after 0, 6, 12, 18 and 24 months of treatment. Hematological parameters (hemoglobin, RBC, platelet count, WBC, packed cell volume, differential count, plasma fibrinogen, thromboplastin time and prothrombin time) and clinical chemistries (sodium, potassium, chloride, calcium, cholesterol, triglycerides, glucose, urea, SGOT, SGPT, alkaline phosphatase and total proteins) were measured after 3, 6, 9, 12, 15, 18, 21 and 24 months; blood acid balance was also measured on treatment day 216. Bone (femur) marrow smears were also obtained after 24 months of treatment. Post-mortem examinations included body weight and organ weights of the heart, liver, kidney, testes and adrenal glands.

After 50 weeks of treatment, dogs treated with polydextrose did not gain body weight, and their body weights were 3-4 kg lower than the control group. Dogs in the polydextrose-treated group gained weight during the 6-month recovery period, although their body weights were less than the control group at time of autopsy. All polydextrose-treated dogs, except for two females, showed variable degrees of anorexia and reduced appetite during treatment, which was absent during the recovery period. Watery diarrhea occurred each morning after polydextrose administration and stopped within 3 days of cessation of treatment. Fecal blood was rare indicating that irritation of the GI tract did not occur. Vomiting rarely occurred in any of the dogs included in the study. Estrus periods in female dogs were unaffected by polydextrose treatment. Ophthalmic examinations showed no treatment-related eye lesions. Plasma sodium, potassium, chloride, glucose, protein, SGOT, SGPT and triglyceride concentrations, as well as alkaline phosphatase, were unaffected by polydextrose treatment. Blood pH, pCO<sub>2</sub> and HCO<sub>3</sub><sup>-</sup> measured on treatment day 216 were also unaffected. Although plasma calcium concentrations were reportedly increased in polydextrose (neutral form)-treated dogs during this period, the variability in the data was so large that meaningful interpretation of the data is impossible. Urea concentration was increased in some animals, mainly in the polydextrose (neutral form)-treated groups. Burdock and Flamm (1999) reported that hematological parameters were "unremarkable". Polydextrose had no effect on organ weights, except for kidney weights, which

were reduced. The reductions in kidney weights were attributed to electrolyte imbalance resulting from chronic severe diarrhea produced by polydextrose (neutral form).

### (3) Summary of Chronic Toxicity

In dogs fed the high dose of 14 g/kg bw/day polydextrose (acidic form) for 13 months, unformed and/or watery stools occurred daily, which in two dogs disrupted sodium homeostasis, leading to increased renal reabsorption of sodium and calcium. Chronic ingestion increased excretion and reabsorption of calcium by the kidneys, which produced a hypercalcemic state that eventually resulted in calcium nephropathy in two dogs. Calcium nephropathy did not develop in the remaining 8 dogs (five female and 3 male) in the high-dose group. In dogs fed the lower dose of 7 g/kg bw/day polydextrose (acidic form), stools were only occasionally watery and there were no effect on serum calcium or urinary sodium concentrations, and calcium nephropathy did not develop.

Polydextrose (neutral form) produced a diarrhetic effect in both chronic studies, even at the lower dose of 4 g/kg bw/day. The chronic diarrhea condition, as well as increased intestinal calcium absorption, resulted in electrolyte imbalances, which over time, produced calcium nephropathy. A review of these data by Newberne et al. (1988) also concluded that the observed changes in serum chemistry were indirect effects of polydextrose secondary to diarrhea.

It is clear from the data that when compared to acidic form polydextrose, the neutral form of polydextrose elicited diarrhea in dogs. Unlike polydextrose (neutral form), the mild diarrhea induced by polydextrose (acidic form) failed to result in calcium nephropathy. Severe diarrhea produced by polydextrose (neutral form) is associated with an electrolyte imbalance that ultimately results in renal damage, which is absent in dogs fed polydextrose (acidic form).

The data indicate that chronic ingestion of 7 g/kg bw/day of polydextrose (acidic form) does not produce diarrhea, but rather only occasionally watery stools. Because the polydextrose product produced by Danisco (Litesse® Two) and proposed for use in infant formula is the acidic form of polydextrose, the safety evaluation with regard to diarrhea is appropriately based on the results of studies of polydextrose (acidic form).

### **e. Carcinogenicity**

#### (1) Mouse

The carcinogenic effect of polydextrose (acidic form) was investigated in male and female CD-1 mice (Burdock and Flamm 1999). From the time of weaning, mice (n=50 per sex per group) were fed either a basal diet or the basal diet mixed with polydextrose (5% or 10%; 7.5 g/kg bw or 15 g/kg bw) for 562 days (18 months). Positive controls received the basal diet only. Body weights were recorded weekly. Food consumption was determined on selected animals 1 day per week for the first 4 weeks, and then it was determined for one dose level per week during each month on a rotating basis. From treatment day 366 and thereafter, food consumption was determined once every 8 weeks. Clinical observations were recorded weekly at time of weighing. Eye examinations were conducted on days 0, 365 and 548. Clinical chemistry (glucose, urea, SGOT, alkaline phosphatase and calcium) and hematology (white blood cell and differential counts) parameters were measured on all moribund animals and surviving animals at the end of the treatment period. Post-mortem body and organ (liver, kidney and testes) weights were recorded from surviving animals. Histological examination was performed on customary tissues

(specific tissues were not reported) in addition to grossly observed tumors and any lesions found at necropsy.

Polydextrose had no effect on survival or food consumption in treated mice. Clinical symptoms and ocular lesions were absent in polydextrose-treated mice. Body weights were unchanged, as well as organ weights except for liver weight, which was reduced by 13% in males fed 5% polydextrose. Liver weights were equivalent to control in female mice fed 5% polydextrose. Post-mortem gross examination did not reveal any treatment-related abnormalities. Hematological and clinical chemistry parameters were normal in polydextrose-treated mice. Incidence of either benign or malignant tumors was normal in mice treated with polydextrose.

## (2) Rat

The carcinogenic effect of polydextrose (acidic form) was also investigated in male and female Sprague-Dawley rats obtained from the F<sub>1</sub> generation of the multi-generation toxicity study (see page 71). Beginning at birth, rats (n=50 per sex per group) were fed either a basal diet or the basal diet mixed with polydextrose (5% or 10%; 5 g/kg bw or 10 g/kg bw) for 775 days (24 months). Positive controls received the basal diet only. Body weights were recorded weekly. Food consumption was recorded weekly for the first 3 months; and then monthly until the study was terminated. At the time of weighing, rats were observed for clinical symptoms. Eye examinations were performed on treatment days 21, 365, 548 and 730. Hematology (hemoglobin, white blood cell, red blood cell, differential count and bone marrow smears) and clinical chemistry (glucose, urea, proteins, SGOT, SGPT, alkaline phosphatase, cholesterol, sodium, potassium, triglycerides and calcium) parameters were measured after 40 days (20 rats per sex), 391 days (10 rats per sex), 560 days (10 rats per sex) and 775 days (all survivors). Post-mortem body and organ (liver, kidney and testis) weights were recorded after the study was terminated on day 775. Histological examinations were performed on the brain, heart, mesenteric lymph node, spleen, stomach, duodenum, ileum, colon, pancreas, liver, pituitary, thyroid, adrenal, lung, kidney, urinary bladder, ovary, testis, epididymis, mammary gland, femoral bone marrow, uterus, prostate, salivary gland, sternum and any grossly observable tumor with regional lymph nodes and any lesion found in tissues not listed above.

General health, survival, food intake and growth were unaffected by polydextrose (acidic form) treatment. Excreted fecal matter appeared soft and dark in rats fed polydextrose. Ocular lesions and mean clinical chemistries were also unaffected by polydextrose. Burdock and Flamm (1999) reported that hemoglobin and RBC were increased in male and female weanling rats fed 10% polydextrose and the WBC decreased in female rats of the same treatment group and age. Hematological parameters were unaffected by polydextrose treatment at the other time points measured. Organ weights were unaffected by polydextrose treatment. Autopsy and histological examinations did not reveal any treatment-related abnormalities. Tumor incidence in the polydextrose-treated groups did not differ from control.

## (3) Summary of Carcinogenic Effects

In mice, the 13% reduction in liver weight observed in male rats only was small, and was in the low dose group (5% polydextrose); thus it is not considered an effect of treatment. In rats treated with 10% polydextrose, the observed changes in RBC (increased in male and females) and WBC (decreased in females) appeared to be benign. Polydextrose did not elicit any observable adverse effects in these studies. Polydextrose did not increase the incidence of tumors

above control in either mice (7.5 or 15 g/kg bw/day polydextrose) or rats (5 or 10 g/kg bw/day polydextrose). The data indicate that polydextrose is non-carcinogenic in rodents.

Because the dose in rodents was based on the percentage of the diet, the dose of polydextrose when calculated as gram per kilogram body weight per day was probably highest when the rodents were young (i.e., low body weights) and lowest when the rats were old (i.e., high body weights), although consumption data were not reported. The carcinogenicity study conducted in rats is particularly relevant because these rats were exposed to polydextrose in utero, during lactation and for the majority of their life span.

#### **f. Reproductive and Developmental Toxicity**

Pfizer, Inc. investigated the reproductive toxicity potential of polydextrose in experimental animals using several protocols that included the dominant lethal assay, Segment I (reproductive), Segment II (developmental), Segment III (perinatal and postnatal) and a multi-generation study (Burdock and Flamm 1999). The results of these studies are described in the following sections.

##### **(1) Dominant Lethal Assay**

The reproductive effect of polydextrose (neutral form) was evaluated using the dominant lethal assay. Male CD-1 mice (8 weeks old; n=15) were gavaged with polydextrose (1 g/kg bw/day) for 7 days. On the 7<sup>th</sup> day of treatment, each male was mated with three females. Females were replaced every 7 days for an 8-week period. Eleven days after removal from a mating cage, each female was autopsied for evidence of dominant lethality (i.e., number of dead versus live implants). Polydextrose had no statistically significant effect on (1) number of pregnancies, (2) total number of implants, (3) total implants per pregnant female, (4) dead implants per pregnant female, (5) percent dead implants or (6) live implants per pregnant female. (Burdock and Flamm 1999)

##### **(2) Segment I (Reproductive) Studies**

The Segment I study was conducted in Sprague-Dawley rats to investigate the effect of polydextrose on fertility and reproductive performance. Male (n=15) and female (n=30) rats were gavaged daily with polydextrose at dose level of 0, 1, 2 or 4 g/day for 79 days before mating for the males and from the 14th day before mating to day 13 post-coitus or throughout gestation. Two females were placed in a cage with one male for ten consecutive nights for mating. Time zero of pregnancy was determined by observation of sperm or copulatory plug.

Male fertility was unaffected by polydextrose treatment (Table 19). Treatment with polydextrose had no effect on the number of inseminations, pregnancy rate, litter size or pup viability. Growth of pups was unaffected by the polydextrose treatment. Mortality during lactation as measured by the 4 day survival index, lactation index and 21 day survival index was also unaffected. The only observable effect in male rats treated with 4 g/day polydextrose was soft stools.

Table 19. Effect of PDX on Fertility in Male Rats

Parameter	Control	PDX (1 g/day)	PDX (2 g/day)	PDX (4 g/day)
Copulation rate	20/26 (77%)	25/26 (96%)	24/28 (86%)	21/28 (75%)
Pregnancy rate	19/20 (95%)	24/25 (96%)	22/24 (92%)	21/21 (100%)
Litter size at birth	14.84±2.93	13.75±3.84	14.23±2.20	13.00±3.35
Live birth index	278/282 (98.6%)	318/330 (96.4%)	310/313 (99.0%)	272/273 (99.6%)
Number of viable pups at birth	14.6±3.71	13.2±3.91	14.1±2.24	13.0±3.37
4 Day survival index	269/278 (96.8%)	316/318 (99.4%)	307/310 (99.0%)	270/272 (99.3%)
Lactation index (day 4 to 21)	268/269 (99.6%)	305/316 (96.5%)	301/307 (98.0%)	265/270 (98.1%)
21 Day survival index	268/278 (96.4%)	305/318 (95.5%)	301/310 (97.1%)	265/272 (97.4%)
Male pup growth*	6.14	5.92	6.15	6.29
Female pup growth	6.15	6.07	6.10	6.43

\*Male and female pup growth is the ratio of mean body weights on treatment day 21 and 1, statistical analysis was not reported  
Source Burdock and Flamm (1999)

In female rats, abnormal clinical signs or behaviors were absent. Mean growth rates were unaffected by polydextrose treatment (Table 20). Growth was slightly delayed during gestation in female rats treated with 2 or 4 g/day polydextrose, and unaffected in the 1 g/day polydextrose group. Although copulation rate was somewhat lower in the 4 g/day polydextrose-treated group, this effect did not appear to be dose-dependent. Pregnancy rate was unaffected by polydextrose treatment. For females killed on day 13 post-coitus, the number of corpora lutea, implantation efficiency, number of implants, embryomortality or mean number of viable fetuses per litter were unaffected by polydextrose treatment (data not shown). For females that delivered pups, the mean length of gestation, mean litter size at birth, number of live pups at birth, mortality during lactation (lactation index) and growth rate were also unaffected (Table 21). Histological examination of fetuses did not reveal any treatment related malformation. Spontaneous malformations were observed that included "kinky tail" syndrome, diaphragmatic hernia, atrophic lungs, slight hydrocephaly, interventricular septum defect, shrunken pericardium and dilatation of left kidney pelvis. These malformations, or lesions, occurred in such a small number of pups that they were not considered by the investigators to be treatment related.

**Table 20. Effect of PDX on Growth and Fertility in Female Rats**

Parameter	Control	PDX (1 g/day)	PDX (2 g/day)	PDX (4 g/day)
GR (day 0/day 15)*	1 13	1 12	1 12	1 13
GR (day 13/day 0)*	1 24	1 22	1 22	1 18
GR (day 0/day15)**	1 12	1 11	1 12	1 15
GR (day 21/day 0)**	1 58	1 60	1 47	1 54
Copulation rate	26/30 (87%)	28/29 (96%)	25/28 (98%)	23/28 (82%)
Pregnancy rate	26/26 (100%)	28/28 (100%)	24/25 (96%)	22/23 (96%)

GR = growth rate, \*day 15 indicates 15 days prior to conception, \*\*growth of littering females, statistical analysis was not reported  
Source Burdock and Flamm (1999)

**Table 21. Effect of PDX on Pups of Littering Females**

Parameter	Control	PDX (1 g/day)	PDX (2 g/day)	PDX (4 g/day)
Gestation length (days)	21 4	21 2	21 7	21 4
Litter size at birth	14 3±3 66	13 8±3 65	12 4±3 06	13 7±2 49
Live birth index	185/186 (99 5%)	178/180 (98 9%)	142/149 (95 3%)	147/151 (97 3%)
Number of live pups at birth	14 2±3 72	13 7±3 97	11 8±3 16	13 4±2 20
Survival index at day 1	185/195 (94 89%)	178/178 (100%)	140/142 (98 6%)	147/147 (100%)
Survival index at day 4	183/185 (98 9%)	176/178 (98 9%)	139/140 (99 3%)	147/147 (100%)
Lactation index	176/183 (96 2%)	175/176 (99 4%)	138/139 (99 3%)	146/147 (99 3%)
Male Growth*	6 36	6 12	6 58	6 40
Female Growth*	6 44	5 70	6 27	6 60

\*Growth is the ratio of the mean body weights at days 21 over 1, statistical analysis was not reported  
Source Burdock and Flamm (1999)

## (3) Segment II (Developmental) Studies

The Segment II study was conducted in Sprague-Dawley rats to investigate the effect of polydextrose on pregnancy and fetal development. Between gestation day 6 and 15, primiparous pregnant-dated female Sprague-Dawley rats (n=20 per group) received polydextrose at doses of 0, 1, 2, or 4 g/day by gastric intubation. Assuming an average body weight of 200 g for a young-adult rat, these doses are equivalent to 5, 10 and 20 g/kg bw/day. On day 20 post-coitus, female rats were killed and the uteri and ovaries were removed and examined for (1) number of corpora lutea, (2) number and position in the uterus of live and dead fetuses, (3) late resorptions, (4) early resorptions and decidual reaction of the uterine mucosa. Uteri were also stained to reveal very early dead implants, and if found, examined for external malformations and weighed. Half of the fetuses were fixed in ethanol, stained and examined for skeletal malformations. The remaining fetuses were examined grossly for external abnormalities and histopathologically for internal abnormalities.

Mortality, nidation rate, number of corpora lutea per pregnancy, number of implantation sites per pregnancy, embryomortality, number of viable fetuses per litter and male per female fetal weights were unaffected by polydextrose treatment (Table 22). Histological examination revealed an absence of treatment related external abnormalities. Although the percentage of internal abnormalities appeared to be increased 5% in the 2 and 4 g/day polydextrose-treated groups compared to control, this was due to dilatation of kidney pelvis and/or uteri and intrathoracic hemorrhage. These occurrence rates were reported to be within historical variation for this strain of rat.

Table 22. Effect of PDX on Pregnancy and Fetal Development in Rats

Parameter	Control	PDX (1 g/day)	PDX (2 g/day)	PDX (4 g/day)
Mortality	0/20 (0%)	1/20 (5%)	0/20 (0%)	0/20 (0%)
Nidation rate*	17/20 (85.0%)	16/19 (84.2%)	17/20 (85.0%)	18/20 (90.0%)
Corpora lutea/pregnancy <sup>†</sup>	14.4±3.18	13.4±3.07	14.9±2.63	15.1±4.04
Implantation site/pregnancy <sup>†</sup>	11.9±2.50	11.0±4.30	11.9±3.93	12.2±3.66
Pre-implantation loss rate	43/245 (17.55%)	38/214 (17.76%)	42/254 (16.54%)	51/271 (18.82%)
Embryomortality	17/202 (8.4%)	8/176 (4.5%)	10/212 (4.7%)	16/220 (7.3%)
VF/litter (mean ± SD)	10.9±2.42	10.5±4.35	11.9±3.93	11.3±3.82
Male fetal weight (mean ± SD) <sup>†</sup>	3.86±0.64	3.86±0.27	3.86±1.27	3.89±0.31
Female fetal weight (mean ± SD) <sup>†</sup>	3.72±0.64	3.69±0.36	3.69±0.24	3.69±0.41
Sex ratio (M/F)	1.08	0.98	1.08	1.32
External abnormalities**	48/185 (25.9%)	25/167 (14.8%)	43/202 (21.2%)	45/204 (22.0%)
Internal abnormalities**	2/92 (2.17%)	2/84 (2.38%)	12/100 (12.0%)	8/103 (7.76%)
Skeletal abnormalities (vertebral bodies)	5/95 (5.4%)	2/84 (2.4%)	8/102 (7.8%)	3/101 (3.0%)
Skeletal abnormalities (delayed ossification)	1/93 (1.1%)	1/84 (1.2%)	8/102 <sup>††</sup> (7.8%)	2/101 (2.0%)

\*The number of females showing implantation sites over the number of females killed on day 20 post coitus, VF/litter = number of viable fetuses per litter, SD = standard deviation, \*\*Total abnormalities per total fetuses examined, <sup>†</sup>Statistical analysis was performed on these parameters, although the procedures used were not reported, <sup>††</sup>Associated with agnathia, absence of palate and spina bifida  
Source: Burdock and Flamm (1999)

A second Segment II study was conducted in New Zealand white rabbits. Female rabbits (n=15 per group) were intubated (oral administration) daily beginning on gestation day with 0, 3, 6 or 12 g/day of polydextrose, equivalent to doses of 1.5, 3 and 6 g/kg bw/day assuming an average body weight of 2 kilograms. Treated rabbits were observed daily for health status, abnormal behavior or reaction to treatment. Maternal body weights were recorded every 3 days. The experiment was terminated on day 28 post-insemination. Uterus and ovaries were removed and number of corpora lutea, number and position in the uterus of live and dead fetuses, late/early resorptions and decidual reaction of the uterine mucosa were recorded. Fetuses were

examined for external malformations and weighed. The skeletal observations were performed for half of the fetuses. The remaining fetuses were examined histopathologically. The effect of polydextrose on stools in the pregnant females was not reported.

Of the surviving females (66), four experienced diarrhea (1 control, 2 mid-dose and 1 high-dose). Burdock and Flamm (1999) did not attribute the occurrence of diarrhea to treatment because rabbits commonly react to stress with diarrhea. Water consumption was increased in polydextrose-treated rabbits; however, water consumption was not recorded quantitatively. Growth of pregnant females was unaffected by polydextrose treatment. Mortality, nidation rate, pregnancy rate, corpora lutea *per* pregnancy, number of implantation sites, embryomortality, fetal weights, placental weights and amniotic fluid weights were also unaffected (Table 23). External abnormalities (hematoceles) were reported by Burdock and Flamm (1999) to be not dose-dependent. In the 6 g/day polydextrose group, two rabbits were described with severe abnormalities (*i.e.*, spina bifida, club feet, atrophic tail and/or absence of tail). Because these occurred at only the mid-dose, these effects were not considered treatment-related. One to two rabbits of this same group presented internal abnormalities that consisted of spina bifida with absence of tail (1), ectopic left kidney (1), dilation of the kidney pelvis (2) or dilatation of cerebral ventricles (1). Of these internal abnormalities, only one fetus had an abnormality that was of the skeletal-type. These internal abnormalities were absent in the 3 and 12 g/day polydextrose-treated groups.

Table 23. Effect of PDX on Pregnancy and Fetal Development in Rabbits

Parameter	Control	PDX (3 g/day)	PDX (6 g/day)	PDX (12 g/day)
Mortality	3/15 (20.0%)	4/15 (26.66%)	0/15 (0%)	1/15 (6.66%)
Nidation rate*	6/12 (50%)	10/11 (90.9%)	12/15 (80%)	11/14 (78.6%)
Pregnancy rate	4/15 (26.66%)	9/15 (60.0%)	11/15 (73.33%)	10/15 (66.66%)
Corpora lutea/pregnancy <sup>†</sup>	12.5±3.70	13.2±2.54	11.8±1.60	12.1±2.02
Implantation site/pregnancy <sup>†</sup>	8.7±2.06	7.8±2.54	7.7±2.93	8.8±3.05
Pre-implantation loss rate	15/50 (30.00%)	49/119 (41.17%)	45/130 (34.16%)	33/121 (27.27%)
Embryomortality	2/35 (5.7%)	NR	4/85 (4.7%)	7/88 (7.9%)
VF/litter (mean ± SD)	8.3±1.50	7.8±2.54	7.4±3.11	8.1±2.81
Fetus weight (mean ± SD) <sup>†</sup>	32.4±4.38	33.5±4.92	30.7±6.26	33.3±5.31
Placental weight (mean ± SD) <sup>†</sup>	6.3±1.06	6.8±1.20	6.0±1.59	6.7±1.17
Amniotic fluid weight (mean ± SD) <sup>†</sup>	2.0±0.89	2.5±1.47	2.2±1.88	2.3±1.46
Internal abnormalities**	0/17	0/35	6/41 (14.63%)	2/39 (5.13%)

\*The number of females with implants per number of females killed, VF/litter = number of viable fetuses per litter, SD = standard deviation, \*\*Total abnormalities per total fetuses examined, <sup>†</sup>Statistical analysis was performed on these parameters, although the procedures used were not reported, NR=not reported  
Source: Burdock and Flamm (1999)

#### (4) Segment III (Perinatal and Postnatal) Study

The Segment III study was conducted in Sprague-Dawley rats to investigate the effect of polydextrose on fetal development during the last third of pregnancy, delivery, lactation, neonatal and postnatal viability, as well as postnatal growth and development. Virgin female rats (65 days old; mean body weight 202 g) were mated, and then administered polydextrose (0, 1, 2, or 4 g/day) beginning on post-coitus day 15, throughout lactation and up to weaning. Female body weights were recorded every 3 days beginning on the third gestation day. External genital tracts were examined beginning on gestation days 10, 11 and 12. Length of gestation, parturition when possible and lactation were also recorded. Uteri were stained on the day each family was killed (on post-coitus day 25 for non-littering females and at time of weaning for littering females) and examined. The following parameters were recorded for each litter: number of newborns (dead or alive), pup body weights at 24 hours, 4 and 21 days, suckling during the first days (in case of agalactia or cleft palate), growth (turning reflex, pinching reflex, grasping reflex,

time of opening of external auditory meatus, pinna reflex and time of opening of palpebral fissures), and ophthalmology. For five pups per sex per dose, gross necropsy examinations were performed on the first weaning day.

Treatment-related adverse symptoms or abnormal behavior were absent in polydextrose-treated rats. Nidation and parturition rates, as well as gestation length, were unaffected by polydextrose treatment (Table 24). The number of viable pups was reduced in the 2 g/day polydextrose treatment group. Burdock and Flamm (1999) reported this effect to be unrelated to treatment and within biological variation. The live birth index, 4 day survival index and lactation index were also unaffected by polydextrose treatment. The 21-day survival index was dose-dependently increased in polydextrose-treated rats.

**Table 24. Effect of PDX on Maternal and Postnatal Development in Rats**

Parameter	Control	PDX (1 g/day)	PDX (2 g/day)	PDX (4 g/day)
Nidation rate	17/20 (85.0%)	18/19 (95.0%)	18/19 (95.0%)	17/20 (85.0%)
Parturition rate	17/17 (100%)	17/18 (94%)	17/18 (94%)	16/17 (94%)
Gestation length (days)	21.5	20.8	21.2	21.2
Number of viable pups at birth	10.6±3.22	11.6±2.32	9.5±3.61*	12.5±2.20
Number of viable pups at 4 days	10.3±3.39	11.4±2.29	9.4±3.70*	12.5±2.17
Number of viable pups at 21 days	10.1±3.56	11.2±2.28	9.2±3.83*	12.5±2.17
Live birth index	181/182 (99.45%)	197/197 (100%)	161/161 (100%)	188/192 (97.92%)
4 Day survival index	175/181 (96.69%)	194/197 (98.48%)	159/161 (98.76%)	187/188 (99.47%)
Lactation index	171/175 (97.71%)	194/194 (100%)	157/159 (98.74%)	187/187 (100%)
21 Day survival index	171/181 (94.47%)	191/197 (96.95%)*	157/161 (97.51%)*	187/188 (99.47%)*
GR male pups (mean ± SD)	6.72	6.90	6.80	6.30
GR female pups (mean ± SD)	6.73	6.61	6.59	6.21
*The number of females with implants per number of females killed, VF/litter = number of viable fetuses per litter, SD = standard deviation, Statistical analysis procedures used were not reported Source: Burdock and Flamm (1999)				

The effect of polydextrose on growth in postnatal pups is presented in Table 25. The growth rate in rat pups of polydextrose-treated groups is within 10% of the control, which is also

within the normal biological variation for this parameter. Postnatal development of the pups assessed by the appearance of some reflexes or anatomical modifications was reported to be satisfactory. No difference between polydextrose-treated groups and the control group was observed in the appearance of the righting and pinching reflexes. Burdock and Flamm (1999) reported a "slight delay (about 12 hours)" in the appearance of other criteria. Gross pathological examination did not reveal any treatment-related abnormality.

Table 25. Effect of PDX on Postnatal Growth in Rat Pups

Day After Birth	Gender	Control	PDX (1 g/day)	PDX (2 g/day)	PDX (4 g/day)
1	Male	7.5±0.86	6.7±0.91	7.1±1.16	6.8±0.59
1	Female	7.1±0.82	6.4±0.90	6.6±0.69	6.6±0.61
4	Male	11.2±1.62	10.4±1.17	10.7±2.15	9.9±0.82
4	Female	10.5±1.41	9.8±1.13	9.8±1.19	9.4±0.76
21	Male	50.4±8.06	46.2±7.27	48.3±9.18	42.9±4.23
21	Female	47.8±7.20	42.3±8.40	43.5±8.28	41.10±4.44
1-21*	Male	6.72	6.90	6.80	6.30
1-21*	Female	6.73	6.61	6.59	6.21

\*Calculated as the mean growth rate of pups in grams per gram of body weight at day 1, Statistical analysis procedures used were not reported  
Source: Burdock and Flamm (1999)

#### (5) Multi-Generation Study

A multi-generation study was conducted in Sprague-Dawley rats to investigate the effect of polydextrose (acidic form) beginning with exposure in utero until reproductive age, as well as subsequent effect(s) to offspring (Burdock and Flamm 1999). Polydextrose (5% or 10% of the diet) was fed daily to rats that were 23 days old until termination for the initial generation (F<sub>0</sub>) and during the remainder of life for the following three generations (F<sub>1</sub>, F<sub>2</sub> and F<sub>3</sub>). At the time of mating, rats had been fed polydextrose for 60 days (F<sub>0</sub>) or 70 days (F<sub>1</sub> and F<sub>2</sub>). In each generation, rats were mated with male rats fed the same dose of polydextrose. On the first day after birth, litters of the F<sub>1</sub> generation were culled to ten pups (five *per sex*) and no fostering permitted. The F<sub>2</sub> generation was produced by mating 20 F<sub>1</sub> rats of each sex at each dose level after 70 days of polydextrose treatment. The F<sub>3</sub> generation was produced from the F<sub>2</sub> generation using the same procedure. The F<sub>0</sub> and F<sub>2</sub> generations were killed at the time of weaning. All rats were observed daily for general health and behavior. Body weights and food consumption were determined weekly. After 100 days of treatment, eye examinations were performed on the F<sub>0</sub> generation controls and 10% polydextrose-treated group. At birth, offspring were examined for gross physical abnormalities. The number of live and dead pups in each litter was recorded. Ophthalmoscopic examinations were performed on opening of palpebra and before weaning in 50 pups of the control and 10% polydextrose groups in the F<sub>1</sub>, F<sub>2</sub> and F<sub>3</sub> generations. Gross

necropsy was performed on five weanlings *per sex per dose* in each generation. Reproductive performance assessments included (1) fertility index and (2) mating index. Offspring viability (*i.e.*, survival until weaning) was assessed by recording the gestation index. The lactation index was also determined to assess the ability of dams to breed and feed their offspring. Sex ratios were calculated to estimate possible sex-related toxicity.

The general health of the rats was unaffected by polydextrose treatment. Most rats in all generations exhibited soft and dark stools that lasted a few days after ingesting polydextrose. Diarrhea did not occur in polydextrose-treated rats. Food consumption was reported to be slightly increased in males treated with polydextrose in the F<sub>0</sub> and F<sub>1</sub> generations. One female of the 10% polydextrose group in the F<sub>0</sub> generation died of unknown causes. The offspring of this F<sub>0</sub> female that died were killed and excluded from the study. Copulation rate and mating behavior were normal. The values of the mating index in the F<sub>0</sub> generation were 85%, 85% and 95% for the control, 5% polydextrose and 10% polydextrose groups. The variances in the mating index were reported to be unrelated to treatment. Mating indices for the F<sub>1</sub> and F<sub>2</sub> generations were 100% in all three groups. Over the three generations of rats, fertility index in polydextrose groups did not fall below the value in the control group, and gestation length was normal (Table 26).

**Table 26. Effect of PDX on Fertility Index and Gestation Length in Rats**

Parameter	Generation	Control	PDX (5%)	PDX (10%)
Fertility index*	F <sub>0</sub>	85%	90%	95%
Fertility index*	F <sub>1</sub>	100%	100%	100%
Fertility index*	F <sub>2</sub>	90%	100%	95%
Gestation length	F <sub>0</sub>	21.3	21.8	21.7
Gestation length	F <sub>1</sub>	21.4	21.8	21.4
Gestation length	F <sub>2</sub>	21.5	21.8	21.6
*Reported as a percentage only. Statistical analysis procedures used were not reported Source Burdock and Flamm (1999)				

The number of live pups per litter, the number of stillbirths, 4-day survival index, lactation index and 21-day survival index were also unaffected by polydextrose treatment (Table 7). Burdock and Flamm (1999) reported that mean body weights were unaffected by polydextrose treatment in any of the three generations of offspring. Mortality was absent in the F<sub>1</sub> generation and only a few deaths occurred in the F<sub>2</sub> and F<sub>3</sub> generations. The 4-day and 21-day survival indices indicated that polydextrose has no effect on mortality in rats. The Wilcoxon rank sum test indicated that the lactation index was "slightly better" in the polydextrose-treated groups than in the control group. Burdock and Flamm also reported that the sex ratios at weaning indicated that there was no sex-related mortality. They also reported that variations between dose levels and generations were due to initial differences on day 1 after birth. Mean body weights at postnatal days 1 and 4 were slightly higher in male and female rats treated with polydextrose

compared to control. At postnatal day 21 (weaning), pup body weights in the 5% and 10% polydextrose-treated groups were similar to control. Examination of stillborn fetuses and autopsies of pups that died during lactation or weaning did not reveal any treatment-related abnormality. Any pathological observations were of congenital origin or residues of embryonic structures. Ophthalmologic examinations also did not reveal any treatment-related ocular lesions.

Table 27. Effect of PDX on Rat Pups

Parameter	Generation	Control	PDX (5%)	PDX (10%)
Live pups/litter at birth (mean)	F <sub>1</sub>	12.9	12.3	13.0
	F <sub>2</sub>	12.9	12.9	13.8
	F <sub>3</sub>	12.9	11.4	13.7
Live pups/litter at day 4 (mean)	F <sub>1</sub>	9.6	10.0	10.0
	F <sub>2</sub>	9.7	9.9	9.9
	F <sub>3</sub>	9.9	9.7	9.9
Live pups/litter at day 21 (mean)	F <sub>1</sub>	9.1	9.9	10.0
	F <sub>2</sub>	8.7	9.0	9.7
	F <sub>3</sub>	9.1	9.4	9.7
Stillbirths*†	F <sub>1</sub>	3/222 (1.4%)	3/225 (1.3%)	0/247 (0%)
	F <sub>2</sub>	6/264 (2.3%)	6/264 (2.3%)	5/281 (1.8%)
	F <sub>3</sub>	5/237 (2.1%)	7/236 (3.0%)	2/262 (0.8%)
4-Day survival index*	F <sub>1</sub>	96.43%	100.00%	100.00%
	F <sub>2</sub>	100.00%	99.49%	99.50%
	F <sub>3</sub>	98.98%	97.28%	98.95%
Lactation index*†	F <sub>1</sub>	94.44%	99.42%	100.00%
	F <sub>2</sub>	89.74%	92.31%	97.99%
	F <sub>3</sub>	92.13%	97.76%	98.95%
21-Day survival index*	F <sub>1</sub>	91.07%	99.42%	100.00%
	F <sub>2</sub>	89.74%	91.84%	97.46%
	F <sub>3</sub>	91.11%	95.11%	97.18%
Sex ratios at weaning	F <sub>1</sub>	1.04	1.02	1.02
	F <sub>2</sub>	1.08	1.04	1.14
	F <sub>3</sub>	1.08	1.08	1.26
Male body weights (g) at day 1 (mean)	F <sub>1</sub>	6.8	7.1	7.2
	F <sub>2</sub>	6.5	6.9	6.7
	F <sub>3</sub>	6.7	6.9	6.8
Male body weights (g) at day 4 (mean)	F <sub>1</sub>	10.1	10.7	11.3
	F <sub>2</sub>	10.1	10.8	10.3
	F <sub>3</sub>	10.2	10.7	10.9
Male body weights (g) at day 21 (mean)	F <sub>1</sub>	46.2	47.0	46.7
	F <sub>2</sub>	39.2	41.4	36.2
	F <sub>3</sub>	45.6	45.6	44.3
Female body weights (g) at day 1 (mean)	F <sub>1</sub>	6.6	6.7	6.8
	F <sub>2</sub>	6.2	6.6	6.4
	F <sub>3</sub>	6.3	6.7	6.4

Parameter	Generation	Control	PDX (5%)	PDX (10%)
Female body weights (g) at day 4 (mean)	F <sub>1</sub>	10.0	10.2	10.8
	F <sub>2</sub>	9.8	10.5	10.0
	F <sub>3</sub>	9.8	10.3	10.4
Female body weights (g) at day 21 (mean)	F <sub>1</sub>	45.9	44.8	44.8
	F <sub>2</sub>	37.8	39.9	36.0
	F <sub>3</sub>	44.6	44.0	43.1

\*Reported as a percentage only, †Statistical comparisons were performed using the Wilcoxon rank sum test, Statistical analysis was performed on these parameters, although the procedures used were not reported  
Source: Burdock and Flamm (1999)

#### (6) Summary of Reproductive and Developmental Toxicity

Although none of these studies administered polydextrose to lactating pups directly, the data are useful because some of the endpoints measured are relevant and sensitive indicators of toxicity. The growth of neonatal pups whose dams were administered polydextrose (Segment III and multi-generation studies) was either unchanged from control or enhanced (see multi-generation study). Any effect on growth is likely to be detected in the multi-generation study because of the long duration of exposure in successive generations. The data suggest that polydextrose does not interfere with the absorption of dietary nutrients.

Malformations, especially skeletal, are sensitive toxicological endpoints because these occur only rarely in rats. Thus, even small, dose-dependent increases in the frequency of malformations of fetuses may establish a teratogenic effect. Soft tissue and skeletal malformations that could be attributed to polydextrose treatment did not occur in any of the reproductive and developmental studies. This sensitive endpoint provides additional supportive evidence that polydextrose is non-toxic in rodents at the doses administered (10 g/kg bw for rats).

The only significant treatment-related effect observed in these reproductive and developmental studies was softening of the stools. This effect was not severe enough to result in a malnourished state in rodents at the doses tested. No diarrhea was reported in any of the rats receiving polydextrose in any generation. Overall, these reproductive and developmental data indicate that polydextrose is neither a reproductive nor a developmental toxicant at the doses tested.

#### g. Summary of Preclinical Safety Studies

Numerous preclinical safety studies (acute, subchronic, chronic, carcinogenicity and reproductive/developmental) were conducted to investigate the adverse effects, if any, of polydextrose (acidic and neutral forms) administered by the oral route. Under acute exposure conditions, exceedingly high doses (>18 g/kg bw/day) of polydextrose (acidic and neutral forms) were required to produce overt signs of toxicity and mortality. Thus, polydextrose presents very low toxicity when administered acutely. The only consistent observable effect in all three species at these very high doses was diarrhea, which lasted for less than 24 hours post-administration.

The occurrence (i.e., number of animals affected in each group) and severity of diarrhea was dose-dependent.

Feeding rats 10 g/kg bw/day polydextrose (acidic form) for three months (i.e., subchronic) did not produce any observable adverse effect in rats or monkeys. In dogs fed 21 g/kg bw/day polydextrose (acidic form) for three months, the only effect observed was an increase in the prevalence of loosely formed and occasionally watery stools, a laxation effect that had no consistent or appreciable effect on serum, urinary clinical chemistries or kidney. A higher dose (31 g/kg bw/day) of polydextrose (neutral form) fed to dogs for three months produced severe diarrhea that disrupted serum electrolyte balance, which in turn, led to calcium nephropathy in two dogs.

In chronic safety studies in dogs fed 14 g/kg bw/day polydextrose (acidic form) for 13 months, unformed and/or watery stools occurred daily, which in two dogs, disrupted sodium homeostasis, leading to increased renal reabsorption of sodium and calcium and eventually calcium nephropathy. Electrolyte imbalance and calcium nephropathy did not occur in the remaining dogs of this high dose group. In dogs fed a lower dose of 7 g/kg bw/day polydextrose (acidic form), stools were only occasionally watery and there was no effect on serum calcium and urinary sodium concentrations and, not surprisingly, calcium nephropathy did not develop. Polydextrose (neutral form) produced a diarrhetic effect in two chronic studies, even at the lower dose of 4 g/kg bw/day. The chronic diarrhea condition, as well as increased intestinal calcium absorption, resulted in electrolyte imbalances, which over time, produced calcium nephropathy. This effect, which occurred only with the neutral form of polydextrose and not with higher doses of the acidic form, is not relevant to assessing the safety of the Litesse® Two product with regard to diarrhea since these product is polydextrose (acidic form).

Data from rodent carcinogenicity studies indicate that polydextrose is non-carcinogenic. Of the reproductive and developmental studies conducted, the Segment III (*u e*, neonatal exposure) and multi-generation studies are particularly relevant to the safety assessment of polydextrose to infants. Growth was unaffected by polydextrose to pups exposed neonatally in these Segment III and multigenerational studies. Lastly, polydextrose is non-teratogenic.

#### (1) Mechanism of Calcium Nephropathy

The investigators proposed that calcium nephropathy observed in dogs fed polydextrose (neutral form) was a result of the treatment-induced diarrhea. The events leading to calcium nephropathy are (1) induction of severe diarrhea that results in (2) fluctuations in extracellular fluid volume producing an electrolyte imbalance, which in turn leads to (3) a hypercalcemic state and increased renal excretion and reabsorption. The increased workload of the kidney in reabsorbing calcium over a prolonged period of time eventually results in calcium nephropathy.

#### (2) Differential Effect of Polydextrose (Acidic and Neutral Forms) on Diarrhea

It is clear from the data that polydextrose neutral form is more potent at eliciting diarrhea in dogs than is polydextrose acidic form. In studies, diarrhea induced by a dose of 4 g/kg bw/day of polydextrose (neutral form) was severe, while only mild diarrhea was induced in dogs fed 14 g/kg bw/day of polydextrose (acidic form) and no diarrhea was produced in dogs fed polydextrose (acidic form) at 7 g/kg bw/day. Polydextrose (neutral form) disrupted electrolyte balance, leading to renal toxicity at a relatively low dose of 4 g/kg bw/day (chronically fed), whereas electrolytes and kidney were unaffected in dogs chronically fed 7 g/kg bw/day

polydextrose (acidic form). Only a very high dose (14 g/kg bw/day) of polydextrose (acidic form) disrupted electrolyte balance and kidney morphology. These data indicate that the two forms of polydextrose (acidic and neutral) have differential effects on diarrhea, electrolyte balance and renal morphology. The severe diarrhea observed in dogs fed polydextrose (neutral form) is likely due to the presence of potassium (1.5%), which is absent in polydextrose (acidic form). The data demonstrate that polydextrose (neutral form) is relatively more toxic (i.e., renal toxicity) than polydextrose (acidic form), at least in dog models. This finding is significant because the polydextrose produced by Danisco that is proposed for use in infant formula (Litesse® Two) is the polydextrose (acidic form). Therefore, the safety assessment of polydextrose produced by Danisco is based on preclinical safety data from rats, dogs and monkeys that were fed acidic form of polydextrose.

The data indicate that chronic ingestion of 7 g/kg bw/day of polydextrose (acidic form) is without any observable adverse effect in experimental animals.

#### **D. Clinical Studies**

The effect of polydextrose in humans has been investigated in numerous clinical studies that include changes in serum lipids, ability to absorb glucose and induction of diarrhea. Of particular importance is whether gastrointestinal absorption is impaired by polydextrose and/or whether acute severe or chronic diarrhea is induced. Impaired absorption and certain diarrhetic states are effects that can potentially result in impaired health of the infant. The results of these clinical studies are critically evaluated in the following sections.

##### **1. General Safety**

Jie et al. (2000) investigated the effect of polydextrose in healthy male (n=16-17 per group) and female (n=13-14 per group) volunteers (average age of male and female subjects were 32.9 and 29.4 years, respectively). Subjects ingested 0 (control), 4, 8 or 12 grams polydextrose/day for 28 days. After 7, 14, 21 and 28 days of polydextrose supplementation, clinical chemistries and body weights were measured and recorded. Glucose tolerance and glycemic index were determined 7 days before and 28 days after polydextrose supplementation. At the end of the 28-day period, feces were collected and fecal pH, short-chain fatty acids (SCFAs), microbiota, sterols and moisture were measured. Frequency of defecation, ease of defecation, abdominal distention, abdominal cramps, diarrhea and hypoglycemic symptoms were rated on a scale of 1-10.

Clinical chemistry parameters were unaffected by polydextrose. Glycemic index decreased 12% after 28 days of polydextrose supplementation as compared to baseline. Ratings of abdominal distension did not differ between control and polydextrose supplemented groups. Abdominal cramps, diarrhea, hypoglycemic symptoms or other discomforts did not occur in any of the four groups. Both wet and dry fecal weights increased and fecal pH decreased dose-dependently compared to control. Fecal acetate, butyrate and isobutyrate were dose-dependently increased up to 25%, 50% and 45%, respectively, by polydextrose.

Jie *et al* (2000) concluded that consumption of 4-12 grams of polydextrose/day did not produce any observable adverse effects. Polydextrose intake inhibited excessive glucose absorption from the small intestine and was fermented in the lower gut to produce SCFAs, including butyrate. These investigators also reported the effect of polydextrose on gastrointestinal microbiota; however, they used selective media not specific for bifidobacteria or

lactobacilli. Thus, the reported counts for these microorganisms are erroneous. Furthermore, the methods described cannot enable selection, differentiation and enumeration of *Bacteroides fragilis*, *Bacteroides vulgatus* and *Bacteroides intermedius* in stool samples. Thus, the reported data on *Bacteroides* are also erroneous.

## 2. Serum Lipids

Liu and Tsai (1995) investigated the effect of polydextrose on serum lipids in 50 young volunteers (n=10 per group; age 21 years; body weight 67.7 kg). Volunteers ingested 10 grams of polydextrose daily for 18 days. Serum cholesterol, high-density lipoprotein, low-density lipoprotein and triacylglycerol concentrations were measured prior to starting the study and after 12 and 18 days of polydextrose ingestion. After 12 days of ingesting polydextrose, serum cholesterol and low-density lipoprotein concentrations were unaffected. After 18 days of polydextrose ingestion, serum cholesterol and low-density lipoprotein concentrations were reduced by 10% and 17%, respectively. Serum high-density lipoprotein and triacylglycerol concentrations were unaffected by polydextrose. While the relevance of decreased cholesterol and low-density lipoprotein in adults may have little relevance to infants, these observations were interpreted as beneficial by the investigators (Bittner 2002; Gaw 2002). Thus, the effects of polydextrose on serum lipids are not considered adverse effects.

## 3. Microbiota in Adult Humans

Endo et al. (1991) investigated the effect of polydextrose on microbiota, bacterial enzyme activity, putrefactive products, volatile fatty acids and fecal pH in eight (6 males and 2 females) healthy volunteers (ages 27-46 years) fed a high cholesterol diet (HC; amount of cholesterol consumed 980 mg/day). All eight subjects consumed a low cholesterol diet (LC; 159 mg/day) for 2 weeks, then the HC diet for 2 weeks, followed by a HC diet supplemented with polydextrose (15 g/day or 0.25 g/kg bw/day) for 2 weeks. Food records of energy and nutrient intake were obtained over a 5-day period. Fecal specimens were collected from each subject during the last 6 days of each dietary period. All subsequent stools were collected for determination of 24-hour fecal output. Fecal pH, stool weight, microbial growth, putrefactive products (indole, skatol, p-cresol and phenol), volatile fatty acids (iso-valeric acid and iso-butyric acid) and enzyme activity ( $\beta$ -glucuronidase,  $\beta$ -glucosidase, nitroreductase and tryptophanase) were measured.

Table 28 presents the energy and macroingredient intakes of each of the three diets. Carbohydrate intake was greatest, while fat and cholesterol intake was lowest, in the LC diet. Energy and protein intake did not differ considerably among the three diets. Energy and nutrient parameters did not differ between the HC and HCP diets. Fecal water content did not differ among the three diets. Fecal output was reduced during the HC period (as compared to the LC period), while it was increased during the HCP period. Compared to LC diet, fecal pH increased during the HC period, while it was reduced during the HCP period.

**Table 28. Energy and Macroingredient Intakes in Adult Humans (Mean±SD)**

	LC	HC	HCP
Energy (kcal)	2284±453	2849±228	2588±261
Carbohydrate (% kcal)	70.9±3.6	31.8±2.0	29.9±2.0
Protein (% kcal)	15.9±1.5	14.8±1.0	17.5±0.9
Fat (% kcal)	13.2±2.5	53.5±2.0	52.6±1.4
Animal fat (%)	47.5±10.3	74.3±1.9	78.4±2.5
Plant fat (%)	52.5±10.3	25.8±1.9	21.6±2.5
Cholesterol (mg)	159±38	980±78	1021±83
Polydextrose (g)	--	--	15
SD=standard deviation, LC=low cholesterol diet, HC-high cholesterol diet, HCP=high cholesterol plus polydextrose, (-)=not measured Source Endo et al (1991)			

Both the number and frequency of occurrence of *Clostridium perfringens* and *Enterobacteriaceae* were lower during the HCP diets as compared to the HC diets.  $\beta$ -Glucuronidase, nitroreductase and tryptophanase activities were unaffected by polydextrose supplementation.  $\beta$ -Glucosidase activity was increased during the HCP period as compared to the HC period. Polydextrose reduced the stool concentration of indole, iso-valeric, p-cresol and iso-butyric acid (quantitative data provided, mean values were not given).

Endo *et al.* (1991) proposed that polydextrose has a protective effect by inhibiting the growth of *C. perfringens*, which are considered pathogenic bacteria. The reduced putrefactive products by polydextrose is another indicator of a protective effect because these substances are considered to be potential promoters or mutagens produced by *Clostridium*. Members of *Clostridium* group modify bile acids and this modification might represent biotransformation of these compounds into carcinogenic agents. The putrefactive products, such as indole, skatol, p-cresol, and phenol produced from amino acids by *Clostridium*, *E. coli*, *Bacteroides*, *Ruminococcus*, and *Megasphaera* are known as promoters or mutagens. High fiber intake may inhibit the growth of some fecal clostridia, leading to decrease promoters and mutagens. Although, the study by Endo *et al.* is limited by the small number of participants, the short duration of feeding and application of only a single dose of polydextrose, the gastrointestinal changes observed in this study are not considered an adverse effect.

#### 4. Mineral and Glucose Absorption

Although the effect of various dietary fibers on mineral absorption has been reported in infant formulas (Davidsson *et al.* 1996; Bosscher *et al.* 2001; Bosscher *et al.* 2003), the effect of polydextrose on mineral absorption has not been reported in the scientific literature. One study was found in the literature that reported the effect of polydextrose on glucose absorption in humans.

Bamba et al. (1993) investigated the effect of polydextrose on glucose absorption in five healthy volunteers. A perfusion technique was used to measure the glucose absorption rate of volunteers orally administered a polydextrose-free solution followed by a 5% polydextrose solution. The thickness of the unstirred water layer of the upper jejunum was also measured. Glucose absorption and the thickness of the jejunum unstirred water layer were unaffected by polydextrose perfusion. Bamba et al., proposed that the lack of an effect on the unstirred water layer thickness was probably due to the inability of polydextrose to alter the viscosity of the perfused solution. In this single study, the data indicate that glucose absorption is unaffected by polydextrose.

## **5. Laxation**

Several studies have been published in which polydextrose was administered to adults or children (Nakagawa et al. 1990; Liu et al. 1994; Zhong et al. 1998; Flood et al. 2004). Additionally, Flood et al. (2004) reported results of nine unpublished studies in which the effect(s) of polydextrose was investigated in human subjects, including two studies in children. Both of these studies are potentially important in this safety evaluation because gram quantities of polydextrose were consumed. All clinical studies were critically evaluated for the determination of an appropriate no-observable-adverse-effect (NOAEL) level for diarrhea. Table 29 presents the effect of polydextrose on laxation in adults and children from all known clinical studies reported in the literature.

MJ conducted a clinical study (Ziegler et al. 2007) in which polydextrose was given to infants, along with other prebiotic ingredients, as a component of their formula; since this study included other prebiotics in addition to polydextrose, it is described in detail in the first volume of this submission rather than in this volume devoted to polydextrose. Healthy newborn infants received control formula or formula containing either 2 g/L of polydextrose and 2 g/L of galactooligosaccharides (GOS), or 4 g/L of polydextrose, 2.7 g/L of GOS, and 1.3 g/L of lactulose, for 4 months. The infants receiving the formula containing prebiotics had softer and looser stools but were not significantly more likely to experience diarrhea.

Table 29. Effect of polydextrose (PDX) on laxation in adults and children

Gender	Age (years)	Study Design	# of Subjects	Dose(s)		Duration (weeks)	Laxation Effect	Reference
				g/day	g/kg bw/day			
M	21-59	Single-blind	20	0 or 75/150	0, 1 25/2 50	3	Five and 11 cases of diarrhea occurred in the control and PDX groups	Alter (1974)
M	21-54	Double-blind	57	0, 35 or 75	0, 0 58 or 1 25	2	Two cases of diarrhea occurred 35 g/day PDX group Diarrhea did not occur in the control or 75 g/day PDX group	Knirsch (1974)
M&F	21-47	Double-blind	21	40-130	0 67-2 17	1 4	Diarrhea did not occur in any of the subjects that ingested PDX Lowest maximum tolerated dose for laxation was 50 g/day The mean laxative threshold dose was 90 g/day	Raphan (1975a)
M&F	12-60	Double-blind	51	0 or 30/45/60	0 or 0 5/0 75/1	12	Six and 21 cases of softer stools/mild diarrhea occurred in the control and PDX groups	Raphan (1975b)
M&F	41-67	Double-blind	10	0 or 50	n/a	<1	One case of diarrhea occurred in the control and PDX group, respectively	McMahon (1974)
M&F	18-23	Double-blind	16	0 or 30/40/50	0 or 0 5/0 67/0 83	6	Diarrhea did not occur in any of the subjects that ingested PDX	Scrimshaw and Young (1977)
M	20-30	NR	12	0 or 30	0 or 0 5	30	Softer stools and flatulence occurred Weekly fecal mass was increased only 0 02% No effect on stool transit time or stool frequency Diarrhea did not appear to occur	Tomlin and Read (1988)
M	21-32	Double-blind	24	0 or 57 6	0 or 0 83	<1	Diarrhea did not occur in any of the subjects that ingested PDX	Beer (1989)
F	19-50	Double-blind	200	0, 10 20, 30 or 40	0, 0 2, 0 6 or 0 8	<1	Diarrhea did not occur in any of the subjects that ingested PDX	Curtis (1991)
F	NR	NR	22	0, 5, 7 or 10	0, 0 1, 0 14 or 0 2	<1	Soft stools were observed in the 7 and 10 g/day PDX groups The frequency and ease of defecation were unaffected	Nakagawa <i>et al</i> (1990)

000331

NR	NR	NR		0 or 10	0 or 0.17	2.6	PDX improved constipation as measured by the frequency of no bowel or partial bowel movement. No other effect was reported.	Liu <i>et al</i> (1994)
NR	NR	NR	90	8-12	0.13-0.2	4	Increased ease of defecation, softening of the feces, stool amount and decreased fecal pH were observed. Acetic, butyric and iso-butyric acids were increased. Lactic acid producing bacteria ( <i>i.e.</i> , <i>Bifidobacteria</i> , <i>Lactobacilli</i> ) were increased, while pathogenic bacteria were suppressed. No other gastrointestinal effect, including diarrhea was reported.	Zhong <i>et al</i> (1998)
M&F	29-30	Double-blind	120	0, 4, 8 or 12	0, 0.07, 0.13 or 0.2	4	Clinical chemistry parameters were unaffected by polydextrose. Glycemic index decreased 12%. Abdominal cramps, diarrhea, hypoglycemic symptoms or other discomforts did not occur. Fecal weights increased and fecal pH decreased dose-dependently. Fecal acetate, butyrate and isobutyrate were dose-dependently increased.	Jie <i>et al</i> (2000)
M&F	2-16	NR	108	0, 15-55	0, 0.5/0.75/1.0	4	Diarrhea did not occur in control, while transient diarrhea occurred in PDX group. Isolated and transient diarrhea occurred on a single day and disappeared spontaneously. In 2-3 year olds, 6 out of 11 experienced diarrhetic episodes and the total number of diarrhetic episodes was 16 out of 308 subject days. For the entire group of children, the total number of transient diarrhetic episodes was 36 out of 1596 subject days. No dose-dependent effect was reported.	Bunde (1975)

g/day=grams /day, g/kg bw/day=grams per kg body weight per day, M=male, F=female, NR=not reported, #=number, Y=yes, N=no, C=corroborative

000332

### a. Adults

Flood et al. (2004) describe the results of nine unpublished studies in which the effect of polydextrose (the form of polydextrose acidic or neutral not specified) was investigated in adults and children (Alter 1974; Knirsch 1974; McMahon 1974; Bunde 1975; Raphan 1975a; Raphan 1975b; Scrimshaw and Young 1977; Beer 1989; Curtis 1991). Each of these nine studies, as reported by Flood et al. (2004) is described and critically evaluated below.

Pfizer, Inc. conducted a Phase I single-blind study in which male volunteers, between the ages 21 and 59 years, consumed polydextrose for 3 weeks (Alter 1974). Subjects ingested daily a chocolate drink supplemented with an unidentified placebo (n=9) or polydextrose (n=20) for 3 weeks. During the first 2 weeks of treatment, subjects ingested 75 g/day of polydextrose; the dose was increased to 150 g/day during the 3rd week of treatment. The dose in g/kg bw/day was not reported; however, if it is assumed that the average body weight was 60 kg, then the dose of polydextrose administered is calculated to be 1.25 g/kg bw/day and 2.5 g/kg bw/day. Feces were analyzed for pH, water retention, total lipids, nitrogen, calcium and potassium were measured weekly. Gastrointestinal transit time was measured during the week prior to initiating treatment, as well as during the first 2 weeks of treatment. Differences between placebo and polydextrose-treated groups were determined using the Student's t-test.

Eleven of the 20 subjects that ingested polydextrose were dropped from the experiment because of diarrhea (five during week 1, three during week 2 and three during week 3). In the placebo group, five of the nine subjects were dropped due to diarrhea that occurred during the third week. It was reported that gastrointestinal symptoms in the polydextrose-treated group were more severe than in the placebo group. Mean fecal lipids, fecal nitrogen and gastrointestinal transit time were unaffected by polydextrose treatment. Mean fecal water content, potassium, calcium and pH declined during the first week of polydextrose treatment, but were unaffected during the second and third weeks of treatment. Although, Alter (1974) concluded that polydextrose was not well tolerated; Flood et al. proposed that the higher number of dropouts in both the polydextrose-treated and placebo groups was possibly due to consumption of milk (i.e., lactose intolerance). It is possible that the polydextrose administered was the neutralized form, which is the more potent and a form of polydextrose more likely to induce diarrhea inducing. Although the strength of this study is the large doses of polydextrose administered, it is a poorly designed study evidenced by the low number of subjects in each group, the relatively short duration of treatment and few doses (only two) administered for unequal lengths of time (1 week versus 2 weeks at each dose level).

Pfizer, Inc. conducted a double-blind study that investigated the effect of polydextrose in male volunteers (Knirsch 1974). Male subjects (n=6-7 per group) between the ages 21 and 54 years consumed either a normal diet or a diet supplemented with polydextrose (0, 35 or 75 g/day) for 2 weeks, excluding weekends. The dose of polydextrose in g/kg body weight/day was not reported; but is calculated to be 0.58 and 1.25 g/kg body weight/day. Polydextrose was ingested during breakfast and lunch. Subjects, who ingested 75 g/day polydextrose received a third portion in gumdrops and cookies during coffee break. Subjects responded daily to a questionnaire on the effects of the diet on eating and bowel movements. Differences between groups were determined using one-way analysis of variance followed by Tukey's post-hoc test.

It was reported that all subjects completed the study and polydextrose was well tolerated. Two subjects, who consumed 35 g/day polydextrose experienced mild diarrhea, while none of

the subjects ingesting 75 g/day polydextrose experienced diarrhea. Softer stools and flatulence occurred in subjects that consumed 35 or 75 g/day polydextrose. The frequency of symptoms was reported to be greater at the higher dose (data not given). No other information regarding this study was described by Flood et al. (2004). The two cases of diarrhea in subjects ingesting 35 g/day polydextrose could be from causes other than polydextrose consumption because diarrhea did not occur in subjects who ingested a dose over two times greater (75 g/day polydextrose).

Pfizer, Inc. conducted two additional studies in which the effect of polydextrose on the gastrointestinal tract was investigated (Raphan 1975a; Raphan 1975b). A double-blind, single three-sided Latin Square study was performed in which male and female volunteers aged 21-47 years ingested increasing doses of polydextrose for 10 days (Raphan 1975a). Subjects (n=7 per group) ingested either polydextrose or a placebo (maltodextrin) in the morning (fruit-flavored drink), at noon (gelatin dessert) and late afternoon (ice cream). The initial dose of polydextrose administered was 40 g/day, which was increased by an additional 10 g/day up to a maximum dose of 130 g/day. The dose of polydextrose was not reported in g/kg body weight/day; however, assuming an average body weight of 60 kg, the initial and maximum doses are calculated to have been 0.67 and 2.17 g/kg body weight/day, respectively. Subjects consumed polydextrose until one of the following three conditions occurred: subject tolerance was exceeded; a laxation endpoint was reached or the predefined maximum dose was administered. Mean maximum doses were analyzed using two-way analysis of variance.

The maximum laxative-tolerated polydextrose dose varied between 50 and 130 g/day and was reported to be dependent on body weight. The mean laxative threshold dose was determined to be 90 g/day or 1.3 g/kg bw/day. Four subjects discontinued polydextrose intake due to flatulence and cramping, which occurred at the threshold dose of 90 g/day. Because 70% of the subjects reached the laxative effect endpoint without discontinuing polydextrose intake, Flood et al. (2004) concluded that these subjects did not reach their maximum tolerated dose.

The long-term effect of polydextrose on the gastrointestinal tract was investigated by Pfizer, Inc. in a double-blind study (Raphan 1975b). Male and female volunteers aged 12-60 ingested either a placebo (n=13 per group) or increasing doses of polydextrose (n=38) for 12 weeks. Placebo and polydextrose were ingested three times a day at mealtime. The dose of polydextrose ingested was initially 30 g/day (first 5 weeks), then increased to 45 g/day (weeks 6-8) and increased again to 60 g/day (weeks 9-12). The dose of polydextrose was not reported in g/kg body weight/day; calculated to be 0.5 (weeks 1-5), 0.75 (weeks 6-8) and 1 (weeks 9-12) g/kg body weight/day. Throughout the study, doses were adjusted to minimize gastrointestinal symptoms. Mean transit time was determined biweekly. Fasting blood and urine samples were collected prior to initiating treatment, biweekly throughout the 12-week study and the day after the last dose was administered. Hematology, clinical chemistry and urinalysis parameters were measured. Differences in blood chemistry parameters between placebo and polydextrose treatment were determined using the Chi-square test, while differences in mean transit times were determined using the Student's t-test.

The incidence of mild gastrointestinal disturbance (ranged from softened stool to mild diarrhea) was 6 (46%) and 21 (55%) in the placebo and polydextrose groups, respectively. Body weight gain was reduced by 0.045 kg/week. Hematology, clinical chemistry and urinalysis

parameters were unaffected by polydextrose ingestion. Flood et al. (2004) did not report the dose at which the mild gastrointestinal disturbances occurred or when this effect occurred.

McMahon (1974) investigated the effect of polydextrose on blood insulin and glucose kinetics in Type 2 diabetics. The study design was double-blinded and subjects served as their own controls. Two males and eight females aged 41-67 fasted for 12 hours and then ingested, on successive days, a solution containing (a) 100 g glucose, (b) 100 g glucose plus 50 g polydextrose, (c) 50 g glucose, (d) 50 g glucose plus 50 g polydextrose or (e) 50 g polydextrose. Blood insulin and glucose concentrations were measured at baseline and after 0.5, 1, 1.5, 2, 3, 4, and 5 hours of glucose/polydextrose ingestion. Blood glucose parameters measured were peak time, peak concentration, area under the curve (AUC) and area above the baseline concentration. Differences between groups (a) and (b), as well as (c) and (d), were determined using the Student's t-test.

Ingestion of 100 g glucose on an empty stomach produced the greatest side effects. Diarrhea occurred on 1 to 2 occasions after ingesting each of the 5 glucose/polydextrose solutions (the total number of occasions was 6). Polydextrose was judged to be well tolerated. Because Type 2 diabetics tend to be overweight (i.e., greater than 60 kg), it is not possible to estimate the dose of polydextrose administered on a g/kg bw/day basis. Flood et al. (2004) did not report whether blood insulin and glucose concentrations were affected by polydextrose. Because glucose was not well tolerated and could have been a confounding factor, the data presented were not suitable for determination of a NOAEL for diarrhea. However, the results indicate that at the dose administered (i.e., 50 g/day), polydextrose did not result in diarrhea in adult humans.

Scrimshaw and Young (1977) investigated the effect of polydextrose on male and female healthy subjects aged 18-23 year in a double-blind metabolic study. Subjects consumed either a control diet (n=6) or a diet supplemented with increasing doses of polydextrose for 6 weeks (n=10 per group). During the first week of the study, subjects ingested 30 grams of polydextrose daily, followed by 40 g/day during the second week and 50 g/day during weeks 3-6. The dose of polydextrose was not reported in g/kg body weight/day; however, assuming an average body weight of 60 kg, the initial and maximum doses are calculated to have been 0.5 (week 1), 0.67 (week 2) and 0.83 (weeks 3-6) g/kg body weight/day. Hematological, clinical chemistry, urinalysis and fecal (total nitrogen, total fat, calcium, potassium, sodium, iron, zinc and water content) parameters were measured in all subjects. Nutrient balance was assessed by comparison of mean urine and fecal calcium, sodium, potassium, iron, nitrogen and zinc to dietary intake levels. Differences among groups were determined using analysis of covariance statistical test.

Two subjects (one control and one polydextrose) experienced diarrhea for two days only. All subjects administered polydextrose experienced increased flatulence and softer stools. Hematological and clinical chemistry parameters were unaffected in the subjects administered polydextrose. Metabolic utilization of calcium, sodium, potassium, iron, zinc and nitrogen were also unaffected. Flood et al. (2004) concluded that, "...polydextrose can be ingested and tolerated without any untoward metabolic and nutritional consequence in healthy adult subjects". Because diarrhea occurred in one subject in both the control and polydextrose groups, these data do not unequivocally demonstrate that polydextrose induces diarrhea in adults. Thus, at the doses administered (i.e., up to 50 g/day), polydextrose did not induce diarrhea in adult humans.

Tomlin and Read (1988) investigated the effect of polydextrose on colonic function in twelve healthy male volunteers (age 20-30 years). Subjects ingested daily a solution containing polydextrose (30 g/day or 0.5 g/kg bw/day) for 30 days. Subjects maintained a diary of their bowel habits (i.e., time of defecation, form and consistency of stools and time of flatulence). Gastrointestinal transit time was measured after subjects ingested a small radio-opaque plastic marker followed by stool collection. The weights of the collected stools were recorded. A 10-day control period prior to polydextrose ingestion was also included. Table 30 presents the effect of polydextrose on weekly fecal mass, stool transit time, stool frequency and stool consistency (i.e., softness). Stool transit time, and stool frequency were unaffected by polydextrose. Weekly fecal mass was slightly increased (0.02%), while stool consistency was softer during ingestion of polydextrose. Although the weekly fecal mass was statistically significant, the increase was small. The softer stools indicate that polydextrose produced a laxative effect. Although Tomlin and Read (1988) did not mention the occurrence of diarrhea, the method of data collection (i.e., diary of bowel habits) would have detected the occurrence of diarrhea if it had occurred. Thus, it is apparent that diarrhea did not occur in this study at the dose of polydextrose administered (i.e., 30 g/day).

**Table 30. Colonic Function in Adult Human Subjects Following PDX Ingestion**

Parameter	Control Period	PDX (30 g/day)
Weekly fecal mass (kg)	1.2 (0.84-1.47)	1.22* (1.07-1.97)
Stool transit time (hr)	53.9 (49.2-69.6)	59.0 (47.1-74.0)
Stool frequency (per week)	7.6 (6.4-8.6)	7.7 (6.3-10.0)
Stool consistency	5.1 (4.4-5.7)	4.6* (3.6-4.9)

Values are mean (range), kg=kilograms, hr=hour, \*Significantly different from control (p<0.05)  
Source: Tomlin and Read (1988)

Beer (1989) conducted a double-blind, randomized study in which 24 fasted males aged 21-32 consumed 0, 1, 2, 3 and 4 candy bars supplemented with 14.4 g polydextrose/bar. The total dose of polydextrose was 57.6 g or 0.82 g/kg body weight assuming an average body weight of 70 kg for males. Breath samples were collected and analyzed for hydrogen before, during (after ingesting each bar successively) and 8 hours after ingesting control or polydextrose containing candy bars. Lactulose (10 g in 15 ml) was ingested by all subjects prior to consumption of candy bars to establish a baseline of breath hydrogen release. Time- and product-dependent differences in breath hydrogen release were determined using two-way analysis of variance. Differences in the reporting of subjective symptoms that occurred at the time of peak breath hydrogen release were determined using Chi-square analysis.

Breath hydrogen release increased maximally 180 minutes after ingesting 2, 3 or 4 bars containing polydextrose. Breath hydrogen release was unaffected in subjects that ingested only one polydextrose bar. Increased flatulence occurred after ingesting 4 bars supplemented with polydextrose. Diarrhea did not occur in subjects that consumed bars supplemented with polydextrose. No additional information regarding this study was reported by Flood et al. (2004). Although, increase in breath hydrogen and flatulence may not support the prebiotic action of polydextrose, the investigators did not provide the details on magnitude of increase in breath hydrogen or flatulence. The results indicate that at the dose administered (57.6 g/day), polydextrose does not elicit a diarrhetic effect in adult humans.

Curtis (1991) performed a double-blind, two-way crossover study in which 200 females aged 19-50 ingested candy bars containing either (1) polydextrose plus caprenin, (2) a conventional sweetener plus fat or (3) conventional sweetener plus caprenin. Eight groups of subjects ingested 1, 2, 3 or 4 candy bars, each containing (1) 10.1 g polydextrose plus caprenin or (2) conventional sweetener plus fat (i.e., control). Symptoms were recorded over a three-day period, after which the procedure was repeated with the crossover diet. A ninth group of subjects ingested (3) caprenin plus conventional sweetener. Over the three-day observation period, subjects filled out a questionnaire that evaluated gastrointestinal symptoms (i.e., gas/wind, bloating, heartburn, overall feeling, belching, cramping/abdominal gas, nausea, urgency and diarrhea). Differences in gastrointestinal symptoms after ingesting the various diets were determined by analysis of variance.

In subjects that ingested three or fewer bars that contained polydextrose, only flatulence was increased compared to control. In subjects that ingested four polydextrose bars (total dose of 40.4 g or 0.8 g/kg bw), flatulence, bloating and urgency (but not diarrhea) were increased above control. The incidence of other gastrointestinal symptoms (i.e., belching, loose stools or diarrhea) were unaffected by polydextrose. Caprenin had no effect on gastrointestinal symptoms. The results indicate that at the dose administered (40 g/day), polydextrose did not induce diarrhea in adult humans.

Nakagawa et al. (1990) investigated the effect of polydextrose on the frequency and feeling of defecation in 22 healthy female volunteers. Subjects drank a solution of polydextrose (0, 5, 7 or 10 g/day) for five successive days weekly (total number of weeks was not given in the abstract). Although soft stools were observed in subjects that drank 7 or 10 g/day polydextrose, the frequency and feeling of defecation were unaffected by ingesting polydextrose at these doses. The results indicate that polydextrose at the dose administered (i.e., up to 10 g/day) did not induce a diarrhea in adult humans.

Liu et al. (1994) investigated the effect of polydextrose on constipation in 50 young volunteers (gender and age not reported). Subjects consumed 10 g/day polydextrose daily for 18 days. Defecation status was recorded for 14 days prior to polydextrose administration and during the 18 days of polydextrose ingestion. Polydextrose was reported to improve constipation as measured by the frequency of no bowel or partial bowel movement. Liu et al., did not report any other effect in subjects that ingested polydextrose. Because diarrhea did not occur and only one low dose was administered, the data were not used for determination of a NOAEL for diarrhea.

Zhong *et al.* (1998) investigated the effect of polydextrose on bowel function in 90 healthy volunteers (gender, age not given). Subjects ingested 8-12 grams of polydextrose daily

for 30 consecutive days. Bowel frequency, ease of defecation and fecal pH were recorded, and short chain fatty acids and fecal microbiota were measured. An increase in the ease of defecation, softening of the feces, increased stool amount and decrease of fecal pH were observed in polydextrose-treated subjects. Acetic, butyric and *iso*-butyric acids were increased after ingesting polydextrose. Lactic acid producing bacteria (*i.e.*, *Bifidobacterium*, *Lactobacillus*) were increased while pathogenic bacteria were suppressed.

#### **b. Children**

Flood et al. (2004) described a tolerance study in children conducted by Bunde (1975). One hundred and eight children aged 2-16 were divided into a placebo (sucrose and maltodextrin) or treated group (increasing doses of polydextrose). The children were classified into five age groups that included 2-3 years (n=11), 4-6 years (n=11), 7-9 years (n=12), 10-12 years (n=12) and 13-16 years (n=12). The dose of polydextrose during the first week of treatment was 0.5 g/kg bw/day, which was increased to 0.75 g/kg bw/day during the 2<sup>nd</sup> week, and increased again to 1.0 g/kg bw/day during the third and 4<sup>th</sup> weeks of treatment. Polydextrose was administered as a sugar substitute at breakfast in a beverage mix; in a beverage mix at lunch, and in ice cream during the evening. Blood and urine were collected on treatment days 0, 14 and 28 for children aged 2-6 years and on day -7, 0, 14 and 28 for the older children. Frequency of bowel movement, loose stools, flatulence, diarrhea, soft stools and constipation were recorded for children administered the placebo or polydextrose. Differences in the frequency of these gastrointestinal changes were analyzed statistically using the Fisher Exact test. Differences in blood chemistry parameters were analyzed using the Chi-Square test.

Two children (one placebo and one treatment) did not complete the study for reasons that were not related to treatment. Diarrhea did not occur in the placebo group, while diarrhea was experienced in the polydextrose-treated group. There were a total of 36 episodes of diarrhea out of 1,596 treatment days. Frequency of diarrhea was greatest in 2-3 year old children (16 episodes of diarrhea out of 308 treatment days or 6 subjects out of 11). Table 31 presents the number of individual episodes that occurred at the highest dose administered. All cases of diarrhea were isolated and transient; occurring on a single day and disappearing spontaneously, despite continued polydextrose treatment. Flatulence was the only side effect that persisted for more than two days. Blood and urine chemistries were normal in all subjects.

Because episodes of diarrhea were transient, it is possible, but not likely, that factors other than polydextrose, such as lactose intolerance, intolerance to consumption of other foods and ingestion of certain medications, contributed to the isolated cases of diarrhea. Flood et al. (2004) concluded, "on a unit-weight basis, children are no more sensitive to low digestible carbohydrates than are adults," which our analysis also supports. Our analysis also indicates that the diarrhea-inducing effect of polydextrose occurs in children when the dose exceeds 1 g/kg bw/day. Therefore, the NOAEL for diarrhea induced by polydextrose in children is determined to be 1 g/kg bw/day.

**Table 31. Effect of PDX on Diarrhea in Children**

Age (years)	# Subjects	Highest Dose (g/day)	Diarrhea Episodes <sup>†</sup>
2-3	11	10-15	4
4-6	11	10-20	1
7-9	12	15-30	1
10-12	12	15-40	4
13-16	12	20-55	1

<sup>†</sup> Individual daily transient episodes (not number of subjects), no chronic diarrhea reported  
Source Flood et al (2004)

### c. Infants

MJ conducted a study (Ziegler et al. 2007) in which polydextrose was given to infants, along with other prebiotic ingredients, as a component of their formula. As this study included other prebiotics in addition to polydextrose, it is described in detail in the first volume of this submission rather than in this volume devoted to polydextrose. In a double-blind, randomized, controlled, parallel-group design, healthy newborn infants were evaluated for growth from 14 to 120 days of age while receiving either control formula or formula containing either 2 g/L of polydextrose (90<sup>th</sup> percentile intake at 0.4 g/kg/bw/day) and 2 g/L of galactooligosaccharide (GOS) (90<sup>th</sup> percentile intake at 0.4 g/kg/bw/day), or 4 g/L of polydextrose (90<sup>th</sup> percentile intake at 0.8 g/kg/bw/day), 2.7 g/L of GOS (90<sup>th</sup> percentile intake at 0.54 g/kg/bw/day), and 1.3 g/L of lactulose. There were no significant differences among the three groups in growth as measured by weight, length, or head circumference. The infants receiving the formula containing prebiotics had softer and looser stools, more typical of breast-fed than formula-fed infants, an expected difference that is regarded as beneficial. The prebiotic-fed infants were not significantly more likely to experience diarrhea or other severe adverse effects attributable to the prebiotic.

Good

## 6. Summary of Clinical Safety Studies

The effects of polydextrose on serum lipid concentration, nutrient absorption and diarrhea have been investigated in numerous clinical studies of children and adults and one published study of infants. The studies demonstrated that polydextrose does not adversely affect serum lipid concentration or glucose absorption. To the contrary, the reduction in serum lipid concentration observed after polydextrose ingestion might be regarded as a health benefit effect. The lack of any effect on glucose absorption by polydextrose is in agreement with animal studies in which it was demonstrated that polydextrose does not interfere with nutrient absorption. Because hematological, clinical chemistry and urinalysis parameters are normal in individuals that ingested up to 50 g/day (i.e., 0.83 g/kg bw/day) polydextrose, the data indicates that polydextrose is non-toxic at this level.

The potential association of high intake levels of polydextrose and diarrhea induction requires careful examination because (1) diarrhea was the only adverse effect observed in experimental animal studies (*i.e.*, dogs and rats) fed polydextrose; and (2) infants are known to be susceptible to dehydration and reduced nutrient intake under certain diarrhetic conditions (Finberg 1972). The data indicate that treatment-related diarrhea does not occur at a dose of 1 g/kg bw/day (highest dose tested) or below in adults. Laxation is reported to occur at doses at or above 0.5 g/kg bw/day; however, laxation is not considered an adverse effect. Based on the results of a double blind, randomized clinical trial in infants (Ziegler et al. 2007), supplementation of formula with a prebiotic mixture including 4 g/L of polydextrose (double the intended addition rate) resulted in normal growth and softer, looser stools. This is certainly within the normal laxation range for infants and actually closer to the values typically reported for breast fed infants. In measures of both 24 hour parental recall and rates of study discontinuation, diarrhea was not significantly higher in the infants receiving the prebiotic formula, even at the higher (8 g/L) supplementation level.

## IV. Safety Assessment/GRAS Determination

### A. Safety of PDX

In this GRAS determination, published ADME, preclinical and clinical studies, as well as safety assessment procedures, were critically evaluated for the determination of whether the proposed use of polydextrose is safe, and GRAS, for human consumption from infant formula. Mead Johnson & Co. USA proposes to use Litesse® Two as an ingredient added to infant formula at the maximum intended addition level of 2.5 g/liter (equivalent to 2 g/L of PDX). A conservative estimate (i.e., 90<sup>th</sup> percentile intake during the highest period of formula consumption) of polydextrose consumption from its proposed use in infant formula for males and females is 0.4 g/kg bw/day.

Experimental data from ADME studies indicate that polydextrose is not stored within tissues, but rather is completely excreted in feces (~60%), expired air (~30%) and urine. Only a minor amount (approximately 12% in rats) of polydextrose is metabolized by gastrointestinal mammalian enzymes within the small intestine. Within the large intestine, polydextrose is subjected to bacterial degradation to CO<sub>2</sub> and volatile short-chain fatty acids, thus providing a prebiotic effect. Because polydextrose contains large molecular weight polymers of randomly joined glucose, a significant portion (~60%) of an orally ingested dose of polydextrose is excreted in the feces unaltered by either mammalian digestive enzymes or bacterial fermentation processes. The elimination and excretion of polydextrose occurs within hours of administration, with a half-life of less than 30 minutes. Because polydextrose is rapidly excreted unchanged or as CO<sub>2</sub>, an innocuous end product, this metabolic profile indicates that polydextrose is unlikely to elicit an adverse biological effect.

The effect of polydextrose on the gastrointestinal tract has been extensively investigated. Polydextrose has been found to have effects on the gastrointestinal system that are regarded as beneficial, including increased calcium absorption and accelerated neonatal GI maturation. Gastrointestinal tract maturation is of particular importance because it prevents pathogens from colonizing, thus polydextrose may have a protective effect to the gastrointestinal system in infants. Polydextrose does not interfere with normal gastrointestinal transport mechanisms or absorption of glucose, minerals or lipids. Polydextrose also does not adversely affect microbiota of the lower gastrointestinal tract. Collectively, the data indicate that ingestion of polydextrose up to 1 g/kg/day, more than double the 90<sup>th</sup> percentile intake estimated to result from its intended use in MJ infant formula, does not adversely affect gastrointestinal function.

Numerous published preclinical safety studies (acute, subchronic, chronic, carcinogenicity and reproductive/developmental) have been performed to determine whether oral administration of polydextrose elicits an adverse effect *in vivo*. Under acute oral exposure conditions, exceedingly high doses (i.e., >18 g/kg bw/day) of polydextrose are required to elicit an adverse effect, thus, polydextrose is of low toxic potential. Data from subchronic and chronic safety studies in rats and dogs indicate that the only consistent and appreciable biological effect resulting from ingestion of polydextrose (acidic form) at doses that are equal to or exceed 14 g/kg bw/day is loose to watery stools. In a sensitive species, such as the dog, chronic diarrhea (i.e., persistent over many months to years), can lead to nephrotoxicity. However, nephrotoxicity, or any other adverse effect, is absent in dogs chronically fed polydextrose (acidic form) at a dose of 7 g/kg bw/day. Data from rodent carcinogenicity studies indicate that polydextrose is non-

carcinogenic, non-reproductive toxic and non-teratogenic. Importantly, it was repeatedly demonstrated that polydextrose does not adversely affect growth in rat pups, as shown in Segment III neonatal and multi-generation rodent studies. Neonatal rats and mice received polydextrose in the carcinogenicity studies as well as the multi-generation reproductive/developmental study and no adverse effects were reported for these animals of developmental stages analogous to human infants.

The preclinical safety studies demonstrate that the only biological effect that could be of concern for the infant ingesting polydextrose (acidic form) is diarrhea. In both experimental animals and humans, large doses of polydextrose (acidic form) were required to elicit a diarrhetic response that is associated with any adverse effect. In experimental animals, 14 g/kg bw/day of polydextrose (acidic form), or greater, was required to induce diarrhea. Diarrhea was not observed in adults and children administered polydextrose up to 1 g/kg bw/day. This no-effect dose (i.e., 1 g/kg bw/day) for humans is well in excess of the amount of polydextrose that will be consumed from its proposed use at a concentration of 2 g/L (i.e., 0.4 g/kg bw/day) for males and females.

The results of a recently published double blind, randomized, prospective clinical trial in which infants were fed mixtures of polydextrose, galactooligosaccharides, and lactulose for four months corroborate other published findings demonstrating the safety of polydextrose (Ziegler, et al. 2007). Infants consuming formula supplemented at the highest level of PDX (2X MJN intended addition level) resulted in normal growth and a laxation pattern characterized by softer, looser stools. This is certainly within the normal laxation range for infants and actually closer to the values typically reported for breast fed infants. In measures of both 24 hour parental recall and rates of study discontinuation, diarrhea was not significantly higher in the infants receiving the prebiotic formula.

Based on the long history of use, complete and rapid elimination from the body, lack of any adverse effect observed in preclinical safety studies (including those studies that included neonatal animals), lack of an adverse effect on neonatal growth, no interference with nutrient absorption, no detrimental effect on gastrointestinal microbiota and an expected 90<sup>th</sup> percentile dose (0.4 g/kg bw/day) that is well below the diarrhea inducing dose (>1 g/kg bw/day); the proposed use of Litesse® Two in infant formula at a level of 2.5 g/liter (equivalent to 2 g/L of PDX) of formula is considered safe.

## **B. General Recognition of the Safety of PDX**

The proposed use and use level of polydextrose has been determined to be safe through scientific procedures set forth under 21 CFR 170.30(b). Furthermore, because this safety assessment satisfies the common knowledge requirement of a GRAS determination, this intended use can be considered GRAS.

Determination of the safety and GRAS status of polydextrose for direct addition to infant formula at a level not exceeding 4 g/L (twice the intended level of 2 g/L) was made through the deliberations of an Expert Panel consisting of Dennis M. Bier, M.D, Michael P. Doyle, Ph.D., George C. Fahey, Ph.D., Glenn R. Gibson, Ph.D., Berthold V. Koletzko, M.D., Robert A. Rastall, Ph.D., and John A. Thomas, Ph.D. These individuals are qualified by scientific training and experience to evaluate the safety of food and food ingredients. These experts have carefully

reviewed and evaluated the publicly available information summarized in this document, and have concluded:

*Ingestion of polydextrose from the proposed use results in an intake of polydextrose by infants that remains within safe limits established by published animal and human studies and the history of safe use of polydextrose. Danisco's polydextrose products have been sufficiently characterized to ensure that they are food-grade products. No evidence exists in the available information on polydextrose that demonstrates, or suggests reasonable grounds to suspect, a hazard to infants when polydextrose is added as a dietary ingredient to infant formula at a level of up to 4 g/L.*

It is their opinion that other qualified and competent scientists reviewing the same publicly available data would reach the same scientific conclusion. The Expert Panel thus determined that polydextrose is safe and GRAS for addition to infant formula at up to 4 g/L, with or without combination with other prebiotic ingredients.

Relying on the opinion of the Expert Panel, along with more recent information (such as Ziegler et al. (2007) that was not available to the Expert Panel, MJ concludes that Litesse® Two are safe and GRAS for its intended use as a dietary ingredient to be added to infant formula, along with galactooligosaccharides, at a level not to exceed 2.5 g/L (equivalent to 2 g/L of PDX).

## V. References

- American Academy of Pediatrics (AAP) (1997) Breast feeding and the use of human milk. *Pediatrics* 100:1035-1039.
- Anonymous (1981) Newly approved bulking agent replaces higher calorie ingredients. *Food Development* 15:38-39.
- Anonymous (1993) Physiological factors in feeding studies of polydextrose in animals and man: Discussion. *Toxicology Forum - Annual Summer Meeting*. Given Institute of Pathobiology. p. 286-287.
- Anonymous (site visited on 07/07/2004) Polymer Synthesis. <<http://plc.cwru.edu/tutorial/enhanced/files/polymers/synth/Synth.htm>>.
- Achour, L.; Flourie, B.; Briet, F.; Pellier, P.; Marteau, P. and Rambaud, J.C. (1994) Gastrointestinal effects and energy value of polydextrose in healthy nonobese men. *American Journal of Clinical Nutrition* 59:1362-1368.
- Allingham, R.P. (1982) Polydextrose - a new food ingredient: Technical aspects. In *Chemistry of Foods and Beverages Recent Developments*. Academic Press, New York. p. 293-303.
- Alter, S. (1974) Polydextrose study number 1, CP-31,081 phase 1 study. Pfizer, Inc., Groton, CT 06340. (cited in Flood 2004)
- Ames, B.N.; Lee, F.D. and Durston, W.E. (1973) An improved bacterial test system for the detection and classification of mutagens and carcinogens. *Proceedings of the National Academy of Sciences of the United States of America* 70:782-6.
- Ames, B.N. (1971) The detection of chemical mutagens with enteric bacteria. In *Chemical Mutagens: Principles and Methods for their Detection*. (A. Hollaender, Ed.). Vol. 1. Plenum, New York. p. 267-282.
- Anderson, K.N.(ed.). 2003. *Mosby's medical, nursing, and allied health dictionary, 6<sup>th</sup> Edition*. Mosby Publishing Company, Inc.
- Arturson, G.; Groth, T. and Grotte, G. (1971) Human glomerular membrane porosity and filtration pressure: Dextran clearance data analysed by theoretical models. *Clinical Science* 40:137-158.
- Arturson, G. and Wallenius, G. (1964) The renal clearance of dextran of different molecular sizes in normal humans. *Scandinavian Journal of Clinical and Laboratory Investigation* 16:81-86.
- Ash, M. and Ash, I. (1995) Polydextrose. In *Handbook of Food Additives*. Gower Publishing, Brookfield, VT. p. 668.
- Auricchio, S.; Rubino, A. and Muerstet, G. (1965) Internal glycosidase activities in the human embryo, fetus, and newborn. *Pediatrics* 35:944-954.

- Bamba, T.; Fuse, K.; Chun, W. and Hosoda, S. (1993) Polydextrose and activities of brush-border membrane enzymes of small intestine in rats and glucose absorption in humans. *Nutrition* 9:233-236.
- Beer, W.H. (1989) Gastrointestinal tolerance to multiple confectionery products containing polydextrose. University of Texas Health Science Center, San Antonio, TX. (cited in Flood 2004)
- Beereboom, J.J. (1981) Bulking agents and fillers. In *Impact Toxicol. Food Process*, (Symp). (J. C. Ayres and J. Kirchman, Eds.). Avi, Westport, CT. p. 273-285.
- Behrman, R.E.; Kliegman, R.M.; and Jenson, H.B. (eds.). 2004. Major symptoms and signs of digestive tract disorders. Chapter 287. In *Nelson Textbook of Pediatrics; 17<sup>th</sup> Edition*. Elsevier Science. Philadelphia, Pennsylvania.
- Beynen, A.C., Kappert, H.J. and Yu, S. (2001) Dietary lactulose decreases apparent nitrogen absorption and increases apparent calcium and magnesium absorption in healthy dogs. *Journal of Animal Physiology and Animal Nutrition* 85: 67-72.
- Bill, D.L. (1987) Polydextrose - a new ingredient for low and reduced energy foods. *Proceedings of the Nutrition Society of New Zealand* 12:97-103.
- Bittner, V. (2002) Lipoprotein abnormalities related to women's health. *American Journal of Cardiology* 90:77i-84i.
- Body, L. 2006. Recent advances on structure, metabolism, and function of human milk oligosaccharides. *Journal of Nutrition* 136:2127-2130.
- Bosscher, D.; Van Caillie-Bertrand, M. and Deelstra, H. (2001) Effect of thickening agents, based on soluble dietary fiber, on the availability of calcium, iron, and zinc from infant formulas. *Nutrition* 17:614-618.
- Bosscher, D.; Van Caillie-Bertrand, M.; Van Cauwenbergh, R. and Deelstra, H. (2003) Availabilities of calcium, iron, and zinc from dairy infant formulas is affected by soluble dietary fibers and modified starch fractions. *Nutrition* 19:641-645.
- Budavari, S.; O'Neil, M.; Smith, A.; Heckelman, P. and Obenchain, J. (1999) Polydextrose. *The Merck Index*. Chapman & Hall, Boca Raton, FL. CD-ROM.
- Buddington, R.K. (2001) The use of nondigestible oligosaccharides to manage the gastrointestinal ecosystem. *Microbial Ecology in Health and Disease* 13:9-15.
- Bunde, C.A. (1975) Polydextrose study number VI, CP-31,081. Toleration study in children. Hill Top Research, Miami, OH 45147. (cited in Flood 2004)
- Burdock, G.A. and Flamm, W.G. (1999) A review of the studies of the safety of polydextrose in food. *Food and Chemical Toxicology* 37:233-264.

Burdock, G.A. (1997) Polydextrose. In *Encyclopedia of Food and Color Additives*. Vol. III. CRC Press, Boca Raton, FL. p. 2240-2242.

ChemIDplus (site visited on 05/19/2004) Polydextrose. *National Library of Medicine (NLM)*. <<http://chem.sis.nlm.nih.gov/chemidplus/>>.

Chierici, R., Fanaro, S., Saccomandi, D. and Vigi, V. (2003) Advances in the modulation of the microbial ecology of the gut in early infancy. *Acta Pediatrica* Suppl. 91:56-63.

Choi, Y.S.; Cho, S.H.; Kim, H.J. and Lee, H.J. (1998) Effects of soluble dietary fibers on lipid metabolism and activities of intestinal disaccharidases in rats. *Journal of Nutritional Science and Vitaminology* 44:591-600.

Clydesdale, F.M. (1997) Polydextrose. *Food Additives: Toxicology, Regulation and Properties*. CRC Press, Boca Raton, FL. CD-ROM.

Cooley, S. and Livesey, G. (1987) The metabolizable energy value of polydextrose in a mixed diet fed to rats. *British Journal of Nutrition* 57:235-243.

Craig, S.A.S.; Holden, J.F.; Troup, J.P.; Auerbach, M.H. and Frier, H.I. (1998) Polydextrose as soluble fiber: Physiological and analytical aspects. *Cereal Foods World* 43:370-376.

Craig, S.A.S.; Holden, J.F.; Troup, J.P.; Auerbach, M.H. and Frier, H. (1999) Polydextrose as soluble fiber and complex carbohydrate. In *Complex Carbohydrates in Foods*. (S. S. Cho, Ed.). Marcel Dekker, New York. p. 229-247.

Craig, S.A.S. (2001) Polydextrose: Analysis and physiological benefits. In *Advanced Dietary Fibre Technology*. (B. V. McCleary and L. Prosky, Eds.). Blackwell Science Ltd, Oxford, UK. p. 503-508.

Crockett, M. (1995) Physiology of the neonatal immune system. *Journal of Obstetric, Gynecologic, and Neonatal Nursing* 24:627-634.

Curtis, G.L. (1991) Effect of "light" confections on gastrointestinal tolerability compared to a conventional confection. Harris Laboratories, Inc., Lincoln, NE 68501. (cited in Flood 2004)

Czuba, L.J. (1993) Physiological factors in feeding studies of polydextrose in animals and man. *Toxicology Forum Annual Summer Meeting*. Given Institute of Pathobiology. p. 279-285.

Danisco Sweeteners (2004a) Exhibit "C" - Commercial worksheet prebiotic ingredients. (Personal Communication)

Danisco Sweeteners (2004b) Exhibit "D" - Technical worksheet for prebiotic ingredients. (Personal Communication)

Danisco Sweeteners (2004c) Litesse<sup>®</sup> Microbiological Statement. (Personal Communication)

Danisco Sweeteners (2004d) Litesse<sup>®</sup> The ideal ingredient for creative beverages. (Personal Communication)

Danisco Sweeteners (2004e) Production Process - Litesse<sup>®</sup> Ultra (Polydextrose). (Personal Communication)

Danisco Sweeteners (2004f) Technical Specification. Litesse<sup>®</sup> Ultra. (Personal Communication)

Danisco Sweeteners (site visited on 06/28/2004g) The Litesse<sup>®</sup> family.

<[http://www.daniscosweeteners.com/web/dsw/publicsite/presentation/home/products/litesse/The\\_litessexsupxxregxxxsupx\\_family.html](http://www.daniscosweeteners.com/web/dsw/publicsite/presentation/home/products/litesse/The_litessexsupxxregxxxsupx_family.html)>.

Davidsson, L.; Mackenzie, J.; Kastenmayer, P.; Rose, A.; Golden, B.E.; Aggett, P.J. and Hurrell, R.F. (1996) Dietary fiber in weaning cereals: A study of the effect on stool characteristics and absorption of energy, nitrogen, and minerals in healthy infants. *Journal of Pediatric Gastroenterology and Nutrition* 22:167-179.

Deis, R.C. (2001) Dietary fiber: A new beginning? *Food Product Design* 11:65-79.

Demigné, C., Levrat, M.A. and Rémésy, C. (1989) Effects of feeding fermentable carbohydrates on the cecal concentrations of minerals and their fluxes between the cecum and blood plasma in the rat. *Journal of Nutrition* 119: 1625-1630.

Dinoto, A.; Suksomcheep, A.; Ishizuka, S.; Kimura, H.; Hanada, S.; Kamagata, Y.; Asano, K.; Tomito, F.; and Yokota, A. 2006. Modulation of rat cecal microbiota by administration of raffinose and encapsulated *Bifidobacterium breve*. *Applied and Environmental Microbiology* 72:784-792.

Dow Corning (1994) Anal leakage of 35 CST. Polydimethylsiloxane fluid as affected by various additives in the diet of rats, with cover letter dated 05/09/94. NTIS, Report Number: NTIS/OTS0557409.

Endo, K.; Kumemura, M.; Nakamura, K.; Fujisawa, T.; Suzuki, K.; Benno, Y. and Mitsuoka, T. (1991) Effect of high cholesterol diet and polydextrose supplementation on the microflora, bacterial enzyme activity, putrefactive products, volatile fatty acids (VFA), and pH of the feces in healthy volunteers. *Bifidobacterium Microflora* 10:53-64.

Engfer, M.B. (2000) Human milk oligosaccharides are resistant to enzymatic hydrolysis in the upper gastrointestinal tract. *American Journal of Clinical Nutrition* 71:1589-1596.

Evaldson, G.; Heimdahl, A.; Kager, L. and Nord, C.E. (1982) The normal human anaerobic microflora. *Scandinavian Journal of Infectious Disease, Supplement* 35:9-15.

FCC (2003) Polydextrose. In *Food Chemicals Codex*. 5th Edition. National Academy Press, Washington, D.C. p. 336-339.

FDA (1996a) Current good manufacturing practice, quality control procedures, quality factors, notification requirements, and records and reports, for the production of infant formula; proposed rule. *Federal Register* 61:36153-36219.

FDA (1996b) Inactive ingredient guide. Polydextrose. Food and Drug Administration; Center for Drug Evaluation and Research; Office of Management, Rockville, MD. p. 104.

- Federal Register (1998) Food additives permitted for direct addition to food for human consumption; polydextrose. *Final rule*. 63: 57596-57597.
- Federal Register (2000a) Food additives permitted for direct addition to food for human consumption; polydextrose. *Final rule*. 65: 64604-64605.
- Federal Register (2000b) Food additives permitted for direct addition to food for human consumption; polydextrose. *Final rule*. 65: 79718-79719.
- Figdor, S.K. and Bianchine, J.R. (1983) Caloric utilization and disposition of [<sup>14</sup>C]polydextrose in man. *Journal of Agricultural and Food Chemistry* 31:389-393.
- Figdor, S.K. and Rennhard, H.H. (1981) Caloric utilization and disposition of [<sup>14</sup>C]polydextrose in the rat. *Journal of Agricultural and Food Chemistry* 29:1181-1189.
- Finberg, L. (1972) Dehydration secondary to diarrhea. In *Diagnosis and management*. p. 208-219.
- Flood, M.T.; Auerbach, M.H. and Craig, S.A. (2004) A review of the clinical toleration studies of polydextrose in food. *Food and Chemical Toxicology* 42:1531-1542.
- Fomon, S.J. (1993) Energy intake by normal infants. In *Nutrition of Normal Infants*. Mosby, St. Louis, MO. p. 104-111.
- Fuse, K.; Banba, T.; Sugiyama, K.; Chikamochi, N.; Nakajo, S. and Hosoda, S. (1991) Clinical evaluation of the unstirred water layer influencing digestive and absorptive functions. *Shoka to Kyushu Digestion & Absorption* 14:58-61. (in Japanese)
- Gaw, A. (2002) A new reality: achieving cholesterol-lowering goals in clinical practice. *Atherosclerosis. Supplements* 2:5-8-11.
- Gelardi, R.C. and Mountford, M.K. (1993) Infant formulas: Evidence of the absence of pesticide residues. *Regulatory Toxicology and Pharmacology* 17:181-92.
- Gemmell, R.; Gibson, G. and Rastall, R. (2004) The application of prebiotics to infant/child nutrition and the influence on gut health: Studies with an *in vitro* model (Final Study Report). Unpublished report (information provided by Mead Johnson).
- Glueck, C.J.; Streicher, P.A.; Illig, E.K. and Weber, K.D. (1994) Dietary fat substitutes. *Nutrition Research* 14:1605-1619.
- Goodlad, R.A.; and Wright, N.A. 1983. Effects of addition of kaolin or cellulose to an elemental diet on intestinal cell proliferation in the mouse. *British Journal of Nutrition* 50:91-98.
- Gracey, M. (1982) Intestinal microflora and bacterial overgrowth in early life. *Journal of Pediatric Gastroenterology and Nutrition* 1:13-22.

Grossklaus, R.; Klingebiel, L.; Lorenz, S. and Pahlke, G. (1984) Risk-benefit analyses of new sugar substitutes. 2. The formation of short-chain fatty acids in the ceca of non-adapted and adapted juvenile rats. *Nutrition Research* 4:459-468.

Gudmand-Hoyer, E. and Skovbjerg, H. (1996) Disaccharide digestion and maldigestion. *Scandinavian Journal of Gastroenterology Supplements* 216:111-121.

Gunnar, M.R. (2000) Early adversity and the development of stress reactivity and regulation. In *The Effects of Early Adversity on Neurobehavioral Development. The Minnesota Symposia on Child Psychology*. (C. A. Nelson, Ed.). Vol. 31. Lawrence Erlbaum Associates, Mahwah, NJ. p. 163-200.

Hara, H.; Suzuki, T. and Aoyama, Y. (2000) Ingestion of the soluble dietary fibre, polydextrose, increases calcium absorption and bone mineralization in normal and total-gastrectomized rats. *British Journal of Nutrition* 84:655-661.

Harada, E.; Hashimoto, Y. and Syuto, B. (1995) Polydextrose induces precocious cessation of intestinal macromolecular transmission and development of digestive enzymes in the suckling rat. *Comparative Biochemistry and Physiology. Part A, Physiology* 111:479-485.

Heijnen, A.M., Brink, E.J., Lemmens, A.G. and Beynen, A.C. (1993) Ileal pH and apparent absorption of magnesium in rats fed on diets containing either lactose or lactulose. *British Journal of Nutrition* 70: 747-756.

Huang, L.-L. and Hsu, G.-S. (1996) Effects of dietary fibers and artificial synthetic polysaccharides on apparent absorption of zinc and copper in rats. *Nutritional Sciences Journal* 21:395-408. (in Chinese)

Hirayama, M.; Toyota, K.; Yamada, K. and Hidaka, H. (1990) A convenient method of estimating the intestinal digestibility of saccharides. *Denpun Kagaku* 37:259-262.

Hyun, S.A.; Vahouny, G.V. and Treadwell, C.R. (1963) Effect of hypocholesterolemic agents on intestinal cholesterol absorption. *Proceedings of the Society for Experimental Biology and Medicine* 112:496-501.

Imaizumi, K.; Tominaga, A.; Mawatari, K. and Sugano, M. (1982) Effect of cellulose and guar gum on the secretion of mesenteric lymph chylomicron in meal-fed rats. *Nutrition Reports International* 26:263-269.

IOM (2003) Special considerations for infant formulas. In *Infant formula Evaluating the safety of new ingredients Committee on the evaluation of the addition of ingredients new to infant formula Food and Nutrition Board*. The National Academies Press, Washington, DC. p. 2-8-2-14.

IUPAC (1989) Macromolecular terms relating to individual macromolecules, their assemblies and dilute polymer solutions. *Pure and Applied Chemistry* 61:211.

Jacobs, L.R.; and Schneeman, B.O. 1981. Effects of dietary wheat bran on rat colonic structure and mucosal cell growth. *Journal of Nutrition* 111:798-803.

Jacobs, L.R. and Lupton, J.R. (1986) Relationship between colonic luminal pH, cell proliferation, and colon carcinogenesis in 1,2-dimethylhydrazine treated rats fed high fiber diets. *Cancer Research* 46:1727-1734.

Jana, A.H.; Prajapati, J.P. and Joshi, N.S. (1994) Bulking agents in low-calorie frozen dairy desserts. *Journal of the Society of Dairy Technology* 47:32-38.

JECFA (1987) Evaluation of certain food additives and contaminants. Thirty-first report of the joint FAO/WHO expert committee on food additives. Technical Report Series 759. World Health Organization, Geneva.

JECFA (1998) Compendium of Food Additive Specifications. Food and Nutrition Paper 52. Addendum 6. Polydextroses. Joint FAO/WHO Expert Committee on Food Additives, 51st session, Geneva, Switzerland, 9-18 June 1998. Food and Agricultural Organization of the United Nations, Rome, Italy. p. 103-108.

JECFA Part I. Introduction. Summary of evaluations performed by the Joint FAO/WHO Expert Committee on Food. <<http://jecfa.ilsa.org/section1.htm#52>>.

JECFA (site visited on 07/13/2004a) Polydextroses Modified. Polydextroses (WHO Food Additives Series 16). *WHO Food Additives*. <<http://www.inchem.org/documents/jecfa/jecmono/v16je18.htm>>.

JECFA (site visited on 05/20/2004b) Summary of evaluations performed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Polydextroses. *Joint FAO/WHO Expert Committee on Food Additives*. <<http://jecfa.ilsa.org/evaluation.cfm?chemical=POLYDEX TROSES&keyword=POLYDEX TROSE>>.

Jie, Z.; Bang-Yao, L.; Ming-Jie, X.; Hai-Wei, L.; Zu-Kang, Z.; Ting-Song, W. and Craig, S.A. (2000) Studies on the effects of polydextrose intake on physiologic functions in Chinese people. *American Journal of Clinical Nutrition* 72:1503-1509.

Johnson, M.H. (2001) Functional brain development in humans. *Nature Reviews. Neuroscience* 2:475-483.

Juhr, N.C. and Franke, J. (1992) A method for estimating the available energy of incompletely digested carbohydrates in rats. *Journal of Nutrition* 122:1425-1433.

Kanauchi, O.; Nakamura, T.; Agata, K. and Fushiki, T. (1997) Preventive effect of germinated barley foodstuff on diarrhea induced by water-soluble dietary fiber in rats. *Bioscience, Biotechnology, and Biochemistry* 61:449-454.

Kibbe, A.H. (2000) Polydextrose. In *Handbook of Pharmaceutical Excipients*. 3rd Edition. American Pharmaceutical Association, Washington, DC. p. 389-391.

Kimura, Y.; Nagatani, Y. and Buddington, R.K. (2004) Some dietary fibers increase elimination of orally administered polychlorinated biphenyls but not that of retinol in mice. *Journal of Nutrition* 134:135-142.

- Knirsch, A.K. (1974) Polydextrose study number II. Pfizer, Inc., (cited in Flood 2004)
- Kobayashi, T. and Yoshino, H. (1989) Enzymatic hydrolysis of "polydextrose". *Denpun Kagaku* 36:283-286.
- Kondo, T. and Nakae, Y. (1996) Breath hydrogen and methane excretion produced by commercial beverages containing dietary fiber. *Journal of Gastroenterology* 31:654-658.
- Kotunia, A.; Wolinski, J.; Laubitz, D.; Jurkowska, M.; Rome, V.; Guilloteau, P.; and Zabielski, R. 2004. Effect of sodium butyrate on the small intestine development in neonatal piglets fed by artificial sow. *Journal of Physiology and Pharmacology* 55:59-68.
- Kruger, D.; Ziese, T. and Grossklaus, R. (1990) Caloric availability of polydextrose in rats. *Akt Ernahr Med.* 15:82-84.
- Kunz, C., Rudloff, S., Baier, W., Klein, N. and Strobel, S. (2000) Oligosaccharides in human milk: structure function and metabolic aspects. *Annals of Reviews in Nutrition* 20: 699-722.
- Lebenthal, A. and Lebenthal, E. (1999) The ontogeny of the small intestinal epithelium. *Journal of Parenteral and Enteral Nutrition* 23:S3-S6.
- Lebenthal, E. and Leung, Y.K. (1988) Feeding the premature and compromised infant: gastrointestinal considerations. *Pediatric Clinics of North America* 35:215-238.
- Levanova, L.A.; Aleshkin, V.A.; Vorob'ev, A.A.; Afanas'ev, S.S.; Surikova, E.V.; Rubal'skii, O.V. and Aleshkin, A.V. (2001) Formation of intestinal microflora in children during the first year of their life. *Zhurnal Mikrobiologii i Immunobiologii* 47-50. (in Russian)
- Liebrand, J. and Smiles, R. (1981) Polydextrose for reduced calorie confections. *Manufacturing Confectioner* 61:35-40.
- Liu, S. and Tsai, C.E. (1995) Effects of biotechnically synthesized oligosaccharides and polydextrose on serum lipids in the human. *Journal of the Chinese Nutrition Society* 20:1-12. (in Chinese)
- Liu, S.f.; Ling, Y.s. and Tsai, C.E. (1994) Biotechnically synthesized oligosaccharides and polydextrose reduce constipation and putrefactive metabolites in the human. *Zhonghua Minguo Yingyang Xuehu Zazhi* 19:221-232. (in Chinese)
- Livesey, G.; Johnson, I.T.; Gee, J.M.; Smith, T.; Lee, W.E.; Hillan, K.A.; Meyer, J. and Turner, S.C. (1993) 'Determination' of sugar alcohol and polydextrose absorption in humans by the breath hydrogen (H<sub>2</sub>) technique: The stoichiometry of hydrogen production and the interaction between carbohydrates assessed *in vivo* and *in vitro*. *European Journal of Clinical Nutrition* 47:419-430.
- Lorenz, S. and Grossklaus, R. (1984) Risk-benefit analyses of new sugar substitutes. 1. Nutritional-physiological investigations on osmotic effect and release of glucose in juvenile rats. *Nutrition Research* 4:447-458.

Lupton, J.R.; Coder, D.M. and Jacobs, L. (1988) Long-term effects of fermentable fibers on rat colonic pH and epithelial cell cycle. *Journal of Nutrition* 118:840-845.

Mallett, A.K.; Bearne, C.A. and Rowland, I.R. (1989) The influence of incubation pH on the activity of rat and human gut flora enzymes. *Journal of Applied Bacteriology* 66:433-437.

Marcal Natali, M.R.; Molinari, S. L.; Valentini, L.C.; and de Miranda Neto. M.H. 2005. Morphoquantitative evaluation of the duodenal myenteric neuronal population in rats fed with hypoproteic ration. *Biocell* 29:39-46.

Mazur, A.W.; Mohlenkamp, M.J.; Hiler, G., II; Wilkins, T.D. and Van Tassell, R.L. (1993) Digestibility of selected carbohydrates by anaerobic bacteria. *Journal of Agricultural and Food Chemistry* 41:1925-1930.

McMahon, F.G. (1974) Polydextrose study number III. Tulane University, New Orleans, LA 70118. (cited in Flood 2004)

Meijer, D.K.; Scholtens, H.B. and Hardonk, M.J. (1982) The role of the liver in clearance of glycoproteins from the general circulation, with special reference to intestinal alkaline phosphatase. *Pharm Weekbl Sci* 4:57-70.

Meissner, W.; Schmidt, U.; Hartmann, M.; Kath, R.; and Reinhart, K. 2000. Oral nalaxone reverses opioid-associated constipation. *Pain* 84:105-109.

Mineo, H.; Hara, H.; Kikuchi, H.; Sakurai, H. and Tomita, F. (2001) Various indigestible saccharides enhance net calcium transport from the epithelium of the small and large intestine of rats *in vitro*. *Journal of Nutrition* 131:3243-3246.

Mitchell, H.L. (1996) The role of the bulking agent polydextrose in fat replacement. In *Handbook of Fat Replacers*. (S. Roller and S. Jones, Eds.). CRC Press, New York, NY. p. 235-249.

MJN (2005). Mead Johnson Nutritionals. The Effects of an Infant Formula Supplemented with Prebiotics on Growth and Tolerance in Term Infants. 07/27/2005. Final Study Report, DRC #000842. (Data on File)

Mobassaleh, M.; Montgomery, R.K.; Biller, J.A. and Grand, R.J. (1985) Development of carbohydrate absorption in the fetus and neonate. *Pediatrics* 75:160-166.

Murai, K.; Hisamitsu, K.; Imamura, L. and Kobashi, K. (1994) Effect of oral administration to rats of various undigestible saccharides on fecal pH, water contents and enzyme activities. *Bifidobact Microflora* 13:91-98.

Nafday, S.M.; Chen, W.; Peng, L.; Babyatsky, M.W.; Holzman, I.R.; and Lin, J. 2005. Short-chain fatty acids induce colonic mucosal injury in rats with various postnatal ages. *Pediatric Research* 57:201-204.

- Nakagawa, Y.; Okamatsu, H. and Fujii, Y. (1990) Effects of polydextrose feeding on the frequency and feeling of defecation in healthy female volunteers. *Nippon Eiyo, Shokuryo Gakkaishi* 43:95-101. (in Japanese)
- Nanthakumar, N.N.; Dai, D.; Meng, D.; Chaudry, N.; Newburg, D.S.; and Walker, W.A. 2005. Regulation of intestinal ontogeny: effect of glucocorticoids and luminal microbes on galactosyltransferase and trehalase induction in mice. *Glycobiology* 15:221-232.
- Nelson, A.L. (2001) Special topics. In *High-fiber ingredients*. (A. L. Nelson, Ed.). Eagan Press, St. Paul. p. 83-92.
- Nelson, C.A. (1995) The ontogeny of human memory: A cognitive neuroscience perspective. *Developmental Psychology* 31:723-738.
- Newberne, P.M.; Conner, M.W. and Estes, P. (1988) The influence of food additives and related materials on lower bowel structure and function. *Toxicologic Pathology* 16:184-197.
- Newberg, D.S. (1997) Do the binding properties of oligosaccharides in milk protect human infants from gastrointestinal bacteria? *Journal of Nutrition* 127: S980-S984.
- Ogata, S.; Fujimoto, K.; Iwakiri, R.; Matsunaga, C.; Ogawa, Y.; Koyama, T. and Sakai, T. (1997) Effect of polydextrose on absorption of triglyceride and cholesterol in mesenteric lymph-fistula rats. *Proceedings of the Society for Experimental Biology and Medicine* 215:53-8.
- Oku, T.; Fujii, Y. and Okamatsu, H. (1991) Polydextrose as dietary fiber: Hydrolysis by digestive enzyme and its effect on gastrointestinal transit time in rats. *Journal of Clinical Biochemistry and Nutrition* 11:31-40.
- Osamu, C.; Yukie, M.; Fukio, O. and Yuko, A. (1990) Combined effect of polydextrose and cereal dietary fiber on the growth rate and the cholesterol metabolism in rats. *Chiba Daigaku Engei Gakubu Gakujutsu Hokoku* 7-14. (in Japanese)
- PAFA (1993) Priority-Based Assessment of Food Additives (PAFA). Center for Food Safety and Applied Nutrition. US Food and Drug Administration, Washington, DC. p. 58.
- PDR (2004) Potassium citrate. Physicians Desk Reference. 58<sup>th</sup> Edition. Thompson PDR, Montvale, NJ. Page 2141.
- Probert, H.M.; Apajalahti, J.H.; Rautonen, N.; Stowell, J. and Gibson, G.R. (2004). Polydextrose, lactitol, and fructooligosaccharide fermentation by colonic bacteria in a three-stage continuous culture system. *Applied and Environmental Microbiology* 70:4505-4511.
- Pryde, S.E.; Duncan, S.H.; Hold, G.L.; Stewart, C.S.; and Flint, H.J. 2002. The microbiology of butyrate formation in the human colon. *FEMS Microbiology Letters* 217:133-139.
- Radosta, S.; Boczek, P. and Grossklaus, R. (1992) Composition of polydextrose before and after intestinal degradation in rats. *Stärke* 44:150-153.

- Ranhotra GS, Gelroth JA, Glaser BK. 1993. Usable energy value of selected bulking agents. *J. Food Sci.* 58(5):1176-1178.
- Rao, R. and Georgieff, M.K. (2000) Early nutrition and brain development. In *The Minnesota Symposia on Child Psychology The Effects of Adversity on Neurobehavioral Development*. (C. Nelson, Ed.). Vol. 31. Lawrence Erlbaum Associates, Mahway, NJ. p. 1-30.
- Raphan, H. (1975a) Polydextrose study number IV. Pfizer, Inc., (cited in Flood 2004)
- Raphan, H. (1975b) Polydextrose study number V. Pfizer, Inc., (cited in Flood 2004)
- Rennhard, H.H. (1973) U.S. Pat. 3766165. Patent No. 3766165. (Patent)
- Richter, M.; Grossklaus, R. and Boczek, P. (1994) Enzymatic degradation of polydextrose and of a high-molecular polydextrose fraction. *Starke* 46:27-32. (in German)
- Ruff, H.A. and Rothbart, M.K. (1996) Attention in Early Development. Themes and Variations. Oxford University Press, New York, NY.
- Ruttloff, H.; Noack, R.; Friese, R.; Schenk, G. and Proll, J. (1967) Uber polysaccharidspaltende enzyme im burstensaum der rattenmukosa unter besonderer berucksichtigung der y-amylase. *Acta Biologica et Medica Germanica* 19:831-839.
- Sakata, T. 1986. Effects of indigestible dietary bulk and short chain fatty acids on the tissue weight and epithelial cell proliferation rate of the digestive tract in rats. *Journal of Nutritional Science and Vitaminology* 32:355-362.
- Saku, K.; Yoshinaga, K.; Okura, Y.; Ying, H.; Harada, R. and Arakawa, K. (1991) Effects of polydextrose on serum lipids, lipoproteins, and apolipoproteins in healthy subjects. *Clinical Therapeutics* 13:254-258.
- Savory, C.J.; and Gentle, M.J. 1976. Changes in food intake and gut size in Japanese quail in response to manipulation of dietary fibre content. *British Poultry Science* 17:571-580.
- SCF (2003) Scientific Committee on Food. Report of the Scientific Committee on Food on the revision of essential requirements of infant formulae and in follow on formulae. EU Commission Report SCF/CS/NUT/IF/65, Final, 18 May. Brussels, Belgoum: EuropeanComission, Health and Consumer Protection Directorate-General.
- Schoffen, J.P.F.; Soares, A.; de Freitas, P.; Buttow, N.C.; and Marcal Natali, M.R. 2005. Effects of a hypoproteic diet on myosin-V immunostained myenteric neurons and the proximal colon wall of aging rats. *Autonomic Neuroscience* 122:77-83.
- Schumann, A.; Nutten, S.; Donnicola, D.; Comelli, E.M.; Mansourian, R.; Cherbut, C.; Cortesy-Theulaz, I.; and Garcia-Rodenas, C. 2005. Neonatal antibiotic treatment alters gastrointestinal tract developmental gene expression and intestinal barrier transcriptome. *Physiological Genomics* 23:235-245.

- Scrimshaw, N.S. and Young, V.R. (1977) A clinical metabolic balance study with a polydextrose in young adult subjects. Massachusetts Institute of Technology, Cambridge, MA 02139. (cited in Flood 2004)
- Setser, C.S. and Racette, W.L. (1992) Macromolecule replacers in food products. *Critical Reviews in Food Science and Nutrition* 32:275-297.
- Solomons, N.W. and Rosenthal, A. (1985) Intestinal metabolism of a random-bonded polyglucose bulking agent in humans: *In vitro* and *in vivo* studies of hydrogen evolution. *Journal of Laboratory and Clinical Medicine* 105:585-592.
- Spraycar, M. (1995a) Auscultation. In *Stedman's Medical Dictionary*. 26 Edition. (M. Spraycar, Ed.). Williams & Wilkins, Baltimore, MD. p. 169.
- Spraycar, M. (1995b) Diarrhea. In *Stedman's Medical Dictionary*. 26 Edition. (M. Spraycar, Ed.). Williams & Wilkins, Baltimore, MD. p. 476.
- Spraycar, M. (1995c) Galactorrhea. In *Stedman's Medical Dictionary*. 26 Edition. (M. Spraycar, Ed.). Williams & Wilkins, Baltimore, MD. p. 699.
- Spraycar, M. (1995d) Hematuria. In *Stedman's Medical Dictionary*. 26 Edition. (M. Spraycar, Ed.). Williams & Wilkins, Baltimore, MD. p. 773.
- Spraycar, M. (1995e) Laxation. In *Stedman's Medical Dictionary*. 26 Edition. (M. Spraycar, Ed.). Williams & Wilkins, Baltimore, MD. p. 943.
- Spraycar, M. (1995f) Nephrocalcinosis. In *Stedman's Medical Dictionary*. 26 Edition. (M. Spraycar, Ed.). Williams & Wilkins, Baltimore, MD. p. 1183.
- Spraycar, M. (1995g) Palpation. In *Stedman's Medical Dictionary*. 26 Edition. (M. Spraycar, Ed.). Williams & Wilkins, Baltimore, MD. p. 1285.
- Spraycar, M. (1995h) Prandial. In *Stedman's Medical Dictionary*. 26 Edition. (M. Spraycar, Ed.). Williams & Wilkins, Baltimore, MD. p. 1417.
- Spraycar, M. (1995i) Proteinuria. In *Stedman's Medical Dictionary*. 26 Edition. (M. Spraycar, Ed.). Williams & Wilkins, Baltimore, MD. p. 1443.
- Stappenbeck, T.S.; Hooper, L.V.; and Gordan, J.I. 2002. Developmental regulation of intestinal angiogenesis by indigenous microbes via Paneth cells *Proceedings of the National Academy of Sciences* 99:15451-15455.
- Starck, J.M.; and Rahmaan, G. H. A. 2003. Phenotypic flexibility of structure and function of the digestive system of Japanese quail. *Journal of Experimental Biology* 206:1887-1897.
- Steggerda, F.R. (1968) Gastrointestinal gas following food consumption. *Annals of New York Academy of Sciences* 150:57-66. (cited in Figdor 1983)

- Stenling, R.; Fredrikzon, B.; Nyhlin, H.; Helander, H.F. and Falkmer, S. (1984) Surface ultrastructure of the small intestine mucosa in healthy children and adults: A scanning electron microscopic study with some methodological aspects. *Ultrastructural Pathology* 6:131-140.
- Sugawa-Katayama, Y.; Kondou, F.; Mandai, T. and Yoneyama, M. (1994) Effects of pullulan, polydextrose and pectin on cecal microflora. *Oyo Toshitsu Kagaku* 41:413-418. (in Japanese)
- Tanabe, H.; Ito, H.; Sugiyama, K.; Kiriya, S.; and Morita, T. 2006. Dietary indigestible components exert different regional effects on luminal mucin secretion through their bulk-forming property and fermentability. *Bioscience Biotechnology and Biochemistry* 70:1188-1194.
- Tham, E.; Nathan, R.; Davidson, G.P.; and Moore, D.J. 1996. Bowel habits of healthy Australian children aged 0-2 years. *Journal of Paediatrics Child Health* 32:504-507.
- Thomas, J.W.; Brown, D.L.; Hoch, J.J.; Leary, J.J. and Dokladova, J. (1991) Determination of polydextrose (polymer) and residual monomers in polydextrose by liquid chromatography. *Journal - Association of Official Analytical Chemists* 74:571-573.
- Tomlin, J. and Read, N.W. (1988) A comparative study of the effects on colon function caused by feeding ispaghula husk and polydextrose. *Alimentary Pharmacology and Therapeutics* 2:513-519.
- Trier, J.S. (1968) Morphology of the epithelium of the small intestine. In *Handbook of Physiology: Section 6, Alimentary Canal*. Vol. 3. American Physiological Society, Washington. p. 1125-1175.
- U.S. Department of Health and Human Services (USDHHS), Office of Women's Health (2000) *HHS Blueprint for Action on Breastfeeding* Washington, DC: U.S. Department of Health and Human Services.
- Van Buskirk, N.E. 1984. The review article in MEDLINE: ambiguity of definition and implications for online researchers. *Bulletin Medical Librarians Association* 72:349-352.
- Vandenplas, Y. (2002) Oligosaccharides in infant formula. *British Journal of Nutrition* 87 (Suppl. 2):S293-296.
- Vincent, P.M. (1991) Sugars, sweeteners and EC regulations. 100-124. (Document)
- Walker-Smith, J.A. and Harrison, D. (1966) Small bowel biopsy in children. *Proceedings of the Australian Society of Medical Research* 2:27.
- Walker-Smith, J.A. (1967a) Dissecting microscope appearance of small bowel mucosa in childhood. *Archives of Disease in Childhood* 42:626.
- Walker-Smith, J.A. (1967b) Dissecting microscope appearances of small bowel mucosa in childhood. *Pediatric Research* 1:412.
- Walker-Smith, J.A. (1969) Small bowel morphology in childhood. *Medical Journal of Australia* 1:382-387.

- Walker-Smith, J. (1972) Variation of small intestinal morphology with age. *Archives of Disease in Childhood* 47:80-83.
- Wang, X. and Gibson, G.R. (1993) Effects of the *in vitro* fermentation of oligofructose and inulin by bacteria growing in the human large intestine. *Journal of Applied Bacteriology* 75:373-380.
- Wauben, I.P. and Wainwright, P.E. (1999) The influence of neonatal nutrition on behavioral development: A critical appraisal. *Nutrition Reviews* 57:35-44.
- World Health Organization (WHO) (2002) *The Optimal Duration of Exclusive Breastfeeding Report of an Expert Consultation* Geneva: World Health Organization.
- Wright, J.A.; Gundry, W.; Conroy, R.; Wood, D.; Du Preez, M.; Ferro-Luzzi, A.; Genthe, B.; Kirimi, M.; Moyo, S.; Mutisi, C.; Ndamba, J.; and Potgieter, N. 2006. Defining episodes of diarrhea: results from a three-country study in Sub-Saharan Africa. *Journal Health Population Nutrition* 24:8-16.
- Yoshioka, M.; Doi, R.; Shimomura, Y. and Suzuki, M. (1996) Effects of dietary polydextrose on *in vitro* absorption rate in rats. *Nutrition Research* 16:245-249.
- Yoshioka, M.; Shimomura, Y. and Suzuki, M. (1994) Dietary polydextrose affects the large intestine in rats. *Journal of Nutrition* 124:539-547.
- Yoshioka, M.; Shimomura, Y. and Suzuki, M. (1995) Dietary cellulose improves decreased muscular layer weight of the large intestine in rats fed the diet containing polydextrose. *Nutrition Research* 15:1473-1476.
- Zhong, J.; Zhai, Z.; Jiang, S. and Luo, B. (1998) Studies on effects of polydextrose on the physiological functions of human bowel. *Shanghai Yixue* 21:187-190. (in Chinese)
- Ziese, T.; Kruger, D. and Grossklaus, R. (1995) *In vitro* and *in vivo* inhibition of small intestinal digestion of sugar substitutes by acarbose. *Aktuelle Ernährungsmedizin* 20:229-231. (in German)

**CONCLUSION OF THE EXPERT PANEL:  
GENERALLY RECOGNIZED AS SAFE (GRAS)  
DETERMINATION FOR THE USE OF  
POLYDEXTROSE AS A PREBIOTIC  
INGREDIENT IN INFANT FORMULA**

**Prepared for:  
Mead Johnson & Co.  
Evansville IN**

**August 2005**

**CONCLUSION OF THE EXPERT PANEL:  
GENERALLY RECOGNIZED AS SAFE (GRAS)  
DETERMINATION FOR THE USE OF  
POLYDEXTROSE AS A PREBIOTIC  
INGREDIENT IN INFANT FORMULA**

We, the members of the Expert Panel, have individually and collectively critically evaluated the publicly available information on polydextrose summarized in a monograph prepared by the Burdock Group, as well as other material deemed appropriate or necessary. Our evaluation included review of the starting materials and methods of manufacture of polydextrose; *in vitro*, animal, and human studies of the prebiotic effect and safety of polydextrose; and the safety of consumption of polydextrose by infants in a blend of prebiotic ingredients added to infant formula at the level anticipated by Mead Johnson's intended use. Our summary and conclusion resulting from this critical evaluation are presented below.

- The substance that is the subject of this generally recognized as safe (GRAS) determination is polydextrose, an odorless white to light cream amorphous powder that is a polymer of randomly bonded glucose units with sorbitol end groups and citric acid attached to the polymer by mono- and diester bonds.
- The molecular weight of polydextrose ranges between 250 and 18,000 Dalton with an average of about 1,500 Dalton. The average degree of polymerization (DP) is about 12; approximately 90% of polydextrose polymers have a DP  $\leq 30$ ; while 30% have a DP  $\leq 4$ .
- Polydextrose is prepared by the vacuum-melt condensation method, in which powdered glucose or a glucose-containing material such as hydrolyzed starch is heated under vacuum at 150 to 160°C in the presence of a polyol such as sorbitol and low levels of a polycarboxylic acid such as citric acid. The product may be purified using ion exchange, membrane filtration, or carbon treatment. Food-grade polydextrose meeting JECFA and Food Chemicals Codex specifications is available in powdered form or as an aqueous solution.
- Multiple lots of Danisco Sweeteners' polydextrose products have been analyzed to demonstrate that they consistently meet the physical, chemical, and microbiological specifications that have been established to ensure food-grade material. The powdered form of polydextrose has a shelf life of up to 24 months. The shelf life of aqueous solutions is up to 4 months at ambient temperature or up to 6 months if refrigerated. Polydextrose is also stable when subjected to an acidic environment and high temperature.
- Because of the random glucose-glucose and diester bonds, polydextrose is more resistant to enzyme or acid hydrolysis than other glucose polymers such as in soluble starch and reaches the colon largely intact, were it is fermentable by the microflora.

- MJN intends to add polydextrose to milk-based infant formula at a level not to exceed 4 g/L. This addition may be as one component of a blend of GRAS prebiotic ingredients; the total addition level of any such blend will not exceed 8 g/L of prebiotics.
- The estimated mean daily intake of polydextrose from this use is 0.7 g/kg bw; the estimated 90<sup>th</sup> and 97.5<sup>th</sup> percentiles are 0.8 g/kg bw and 0.9 g/kg bw, respectively. Since there are no other sources of polydextrose in the diets of formula-fed infants, this represents the estimated total daily intake of polydextrose by this population.
- The Food and Drug Administration (FDA) approved polydextrose in 1981 as a direct food additive. Polydextrose is used to replace sugars and as a partial replacement for fat in desserts, confections, chewing gum, baked goods, instant puddings, jams, toppings and frostings, cereal bars, bakery fillings, fruit spreads, salad dressings, cakes and frozen dairy desserts. In 2000, amendments provided for the use of polydextrose as a bulking agent, texturizer, or both in table spreads and as a bulking agent or texturizer in fruit and water ices. Polydextrose is also used as an excipient and as a binder in pharmaceutical formulations. Polydextrose is approved for food use in the European Union and in Japan, China, and other countries in Asia and Latin America.
- In 1981, JECFA evaluated the use of polydextrose in food and established an acceptable daily intake (ADI) of 70 mg/kg bw/day. In 1987, JECFA re-evaluated polydextrose and revised the ADI to "Not Specified," a designation used for food ingredients that have been determined to be of very low toxicity, and for which the total dietary intake resulting from its use does not represent a health hazard to humans.
- The published scientific literature through June 2004 was searched and reviewed. Based on the totality of the evidence found in the published scientific literature, the Expert Panel found that:
  - The absorption, distribution, metabolism, and excretion of polydextrose have been extensively studied in animals and humans. Only a minor amount of polydextrose is digested or absorbed; it is not stored within tissues but is rapidly excreted.
  - Investigations in experimental animals of the effects of polydextrose on the gastrointestinal tract indicates that these effects are generally beneficial, including increased calcium absorption and neonatal maturation.
  - The safety of polydextrose has been studied *in vitro* and *in vivo* in mice, rats, dogs, and monkeys. Research has included acute, subchronic, chronic, multi-generational developmental and reproductive, carcinogenicity, and genetic toxicity studies. Polydextrose does not exhibit any type of toxicity or any gastrointestinal effect other than loose stools at chronic intakes as high as 14 g/kg bw/day. Chronic ingestion of 7 g/kg bw/day of polydextrose is without any observable adverse effect in experimental animals or in humans.
  - Based on numerous clinical studies in children and adults, polydextrose is not toxic in adults at levels of consumption as high as 50 g/day. No adverse effects on microfloral populations, nutrient absorption, or blood biochemistry have been shown.

from consumption of up to 1 g/kg/day by human children or adults. Diarrhea is not seen in children ingesting up to 1 g/kg bw/day.

In addition to the published information cited above, the Expert Panel reviewed the results of a recently completed and not yet published study of polydextrose in human infants. These results, which corroborate the demonstration of safety provided by the published literature, were obtained in a randomized, double-blind, placebo controlled trial in which infants consumed one of three formulas between day 14 and day 120 of life. The control formula was Enfamil® LIPIL® with Iron; the test formulas were the control formula supplemented with 4 g/L of a 50:50 blend of polydextrose and galactooligosaccharides or the control formula supplemented with 8 g/L of a 50:33:17 blend of polydextrose, galactooligosaccharides, and lactulose. While there was some evidence that 8 g/L may represent a tolerance limit for some infants, neither test formula resulted in any adverse effect on growth or any observed safety-related events.

**Conclusion**

We, the undersigned members of the Expert Panel, have individually and collectively critically evaluated the materials summarized above and conclude that:

Ingestion of polydextrose from the proposed uses results in a level of intake that remains within safe limits established by extensive published animal and human studies. Polydextrose has been sufficiently characterized to ensure that it is a food-grade product. Therefore, polydextrose meeting the specifications described in the Food Chemicals Codex is safe when added to milk-based infant formulas at up to 4 g/L as a component of a blend of prebiotic ingredients.

It is also the Expert Panel's opinion that other qualified and competent scientists reviewing the same publicly available information would reach the same conclusion. Therefore, polydextrose is safe, and is GRAS via scientific procedures, when added to milk-based infant formulas at up to 4 g/L.

Dennis M. Bier, M.D.  
Baylor College of Medicine  
Houston Texas, USA

Signature: \_\_\_\_\_  
Date: 12 Sept 2005

Berhold V. Koletzko, M.D.  
University of Munich  
Munich, Germany

Signature: \_\_\_\_\_  
Date: 15.09.2005

Michael P. Doyle, Ph.D.  
University of Georgia  
Griffin Georgia, USA

Signature: \_\_\_\_\_  
Date: 30 September 2005

Robert A. Rastall, Ph.D.  
University of Reading  
Reading, UK

Signature: \_\_\_\_\_  
Date: 21 Sept 2005

George C. Fahey, Ph.D.  
University of Illinois  
Urbana Illinois, USA

Signature: \_\_\_\_\_  
Date: October 6, 2005

John A. Thomas, Ph.D.  
University of Texas (Emeritus)  
San Antonio Texas, USA

Signature: \_\_\_\_\_  
Date: 10/13/05

Glenn R. Gibson, Ph.D.  
University of Reading  
Reading, UK

Signature: \_\_\_\_\_  
Date: 21/9/05

**SUBMISSION END**

000363