Application In Respect of Regulation (EC) 258/97 of the European Parliament and Council Concerning the Proposed Use of Two Phosphated Distarch Products Fibersym®RW & FiberRite® RW as Novel Foods

Applicant:

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Relevant Regulatory Body

ACNFP Secretariat
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SUMMARY

Fibersym® RW and FiberRite® RW are two phosphated distarch phosphate (PDSP) preparations manufactured by MGP Ingredients Inc. (Atchison, Kansas, USA) from wheat starch for use in food.

PDSP is a permitted food additive within the European Union (E1413) and with the exception of foods intended for weaning or young children; its inclusion in food is permitted *quantum satis.* As such PDSP has a long history of safe use within the European Community.

An application for a PDSP product made from maize has been subject to a favourable opinion by the United Kingdom Advisory Committee on Novel Foods and Processes. The Committee's comments have now been forwarded to the European Commission for consideration.

In addition to its technological properties, PDSP is highly resistant to hydrolysis by human alpha-amylases (the enzymes responsible for the primary breakdown of starch to maltose). As such it is considered to be a "resistant starch."

Depending on the basis of their resistivity, resistant starches can be classified as $RS_{1 \text{ through } 4}$ PDSP falls into the category RS_4. Undigested resistant starches (including PDSP) pass through the small intestine into the large intestine where they are broken down by the resident microflora. Metabolites produced by this process include certain small chain fatty acids, which are beneficial to the large intestinal epithelium. As such RS_4 starches in general and PDSP in particular meet the criteria required to satisfy currently accepted definitions of dietary fibre used for labelling purposes.

Permission is sought to include Fibersym® RW and FiberRite® RW as ingredients in a number of farinaceous foods including white bread products, processed breakfast cereals, pasta cakes, biscuits and crackers and starch-based snack foods as an ingredient. The precedent of using resistant starches as sources of additional fibre is already well established both in Member States and elsewhere. For example, within the EU a number of white bread products are now being sold containing high amylose resistant starches. Elsewhere RS-4 products are being used as ingredients in foods already being sold to the public in different countries within the Americas (e.g. Chile and the United States of America) and also in Australia, Japan, South Korea and Malaysia.

Studies commissioned by MGP Ingredients Inc. have demonstrated that at the levels of incorporation proposed, PDSP would not have a detrimental effect on consumer nutrition (based on UK dietary intake data). Other work commissioned by the applicant has shown that the modified starch has no detrimental effects as a consequence of colonic bacterial fermentation and is associated with beneficial effects on the entero-insular axis of humans. These studies supplement existing toxicological data showing PDSP to be safe.

On the basis of the available toxicological information, its equivalence to other (chemically unmodified) resistant starches and level of supplemental phosphate intake; it is proposed that use of either Fibersym® RW or FiberRite® RW would present no additional risk for human health on the bases of their proposed uses in food.

CHAPTER 1: ADMINISTRATIVE DATA

NAME & ADDRESS OF APPLICANT/MANUFACTURER OF PRODUCTS

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CHAPTER 2 GENERAL DESCRIPTION OF NOVEL FOOD

This application is made for the use of a modified starch as a source of dietary fibre in a range of cereal-based foods. The modified starch in question is phosphated distarch phosphate (PDSP). Furthermore the application specifically concerns PDSP produced from wheat starch in accordance with US patents 5 855 946 & 6 299 907 (Seib & Woo, 1999, 2001). This is marketed either as Fibersym® RW or FiberRite® RW (a cooked version of Fibersym® RW). In jurisdictions where they are permitted for use as ingredients these products are referred to as *modified wheat starches*.

Modified starches were described in Commission Directive 95/2/EC (Commission of the European Communities, 1995) as:

Substances obtained by one or more chemical treatments of edible starches which may have undergone a physical or enzymatic treatment and may be acid or alkali thinned or bleached.

Phosphated distarch phosphate (E 1413) was described in Commission Directive 2000/63/EC (Commission of the European Communities, 2000) as:

Starch having undergone a combination of treatments as described for monostarch phosphate and distarch phosphate.

In the same document monostarch phosphate was described as:

Starch esterified with ortho-phosphoric acid or sodium or potassium ortho-phosphate or sodium tripolyphosphate.

While distarch phosphate was described as:

Starch cross-linked with sodium trimetaphosphate or phosphorus oxychloride.

PDSP is permitted for use as a food additive within the European Union as detailed in Commission Directive 2000/63/EC (Commission of the European Communities, 2000). It is listed within Annex I of the directive and can therefore be used *quantum satis* in all foods with the exception of those listed under Article 2 (3) of that directive. With regard to foods covered by the aforementioned article, PDSP is also permitted for use up to a limit of 50g per kg ^{in} weaning foods (Article 2 (3b) and Annex VI of the directive).

When used as an additive, PDSP is generally used as a thickening agent capable of withstanding the shear and other detrimental effects associated with freezing and thawing. It finds uses in a diverse range of foods including pie fillings and soups. Inclusion rates are typically of the order of 2 – 5%.

In nutritional terminology, modified starches such as PDSP are also classed as RS-4 type resistant starches. Although there is no regulatory definition for resistant starches, they are considered to be starches partially or totally resistant to digestion in the small intestine but capable of being partially or totally digested in the colon (Berry, 1986.: Englyst & Cummings 1987). RS-4 type resistant starches are a subgroup, whose resistance is owed to the fact that they have been chemically modified (Baghurst *et al.*, 1996). Generally speaking such starches have been chemically modified to increase resistance against shear and other aspects of food

manufacturing process which disrupt the molecular integrity of the starch molecule. Coincidentally such modifications also result in increased resistance to those α-amylases (enzymes capable of hydrolysing starch) found in the small intestine.

Resistant starches therefore fulfil the criteria set for dietary fibre as set out by organisations such as the American Association of Cereal Chemists (2001); Agence Française de Sécurité Sanité des Aliments (2002), the Health Council of the Netherlands (2006) and the European Food Safety Authority (2007). The dietary fibre content of RS-4 type resistant starches can be determined using recognised and validated analytical methods (AOAC 991.43, Association of Official Analytical Chemists).

MGP Ingredients Inc. wishes to market two PDSP products manufactured from wheat starch (Fibersym® RW (previously known as Fibersym® 70) and FiberRite® RW) as sources of additional dietary fibre for use in a range of cereal-based foods (e.g. bread, biscuits, cakes, pastries breakfast cereals pasta and noodles). In these products the modified starch is used to replace some of the primary source of starch in the recipe – flour.

This is not the first application for PDSP to be considered as a novel food National Starch recently received a favourable opinion concerning the use of a PDSP made from maize starch as a novel ingredient (Food Standards Agency, 2009)

CHAPTER 3 IDENTIFICATION OF ESSENTIAL INFORMATION REQUIREMENTS

Article 1 (2) Regulation (EC) No. 258/97 of the European Parliament and Council dated 27th January 1997 concerning novel foods and novel ingredients states:

This regulation concerns the placing on the market within the Community of foods and food ingredients which have not hitherto been used for human consumption to a significant degree within the community and which fall under the following categories

(a) ... ...
(b) ... ...
(c) foods and food ingredients with a new or intentionally modified primary modified structure ...

Article 3 of the same regulation states:

Foods and food ingredients falling within the scope of this regulation must not
- *present a danger for the consumer,*
- *mislead the consumer*
- *differ from foods or food ingredients which they are intended to replace to such an extent that their normal consumption would be nutritionally disadvantageous for the consumer.*

The modified starches Fibersym® RW and FiberRite® RW meet the description set out in Article 2 (c) of Regulation (EC) 258/97 and must therefore be regarded as novel foods. MGP Ingredients Inc. therefore seeks approval for the use of these modified wheat starches as novel foods and submits this dossier to demonstrate compliance with Article 3 of the same regulation.

This dossier has been constructed in accordance with the guidelines issued by the European Commission in Commission Recommendation 97/618/EC. Section 4 of that document details the scientific classification of novel foods for the assessment of wholesomeness. Within that section, Class 2 foods (complex novel foods from non-GM sources) are described as:

... complex NF[1] which are not, or are derived from sources which have not, been genetically modified. Intact plants animals and micro-organisms used as foods as well as food components (e.g. complex carbohydrates, fats proteins or those substances collectively described as dietary fibre) are included. Two sub classes can be identified:

2.1 the source of the NF has a history of use in the Community
2.2 the source of the NF has no history of food use in the Community

Given that PDSP is used within the Community as a permitted food additive, Fibersym® RW and FiberRite® RW meet the criteria set out within Group 2.1. According to Table II of the guidelines information is required to satisfy the requirements of the following sections of the structured scheme:

1 NF: Novel Foods

I *Specification of the NF*
II *Effect of the production process applied to the NF*
III *History of the organism used as the source of the NF*
IX *Anticipated intake/extent of use of the NF*
X *Information from previous human exposure to NF or its source*
XI *Nutritional Information of the NF*
XII *Microbiological Information on the NF*
XIII *Toxicological Information on the NF*

For each part of the structured scheme within the recommendation the Commission has also provided a set of questions which form the basis of a decision tree.

Answers to the relevant questions addressing the needs of each part of the structured scheme are detailed in Chapter 4 of this submission and further discussed in Chapter 5. References cited anywhere in the submission are listed at the end of Chapter 5.

CHAPTER 4: CONSULTATION OF STRUCTURED SCHEMES (DECISION TREES)

This chapter addresses those sections of the Commission recommendations document 97/618/EC relevant to discussing the safety in use of the modified starches Fibersym® RW and FiberRite® RW. The chapter is sub-divided; each subdivision refers to a specific section in the recommendations document and is numbered accordingly. The chapter is therefore divided as follows

I Specification of the novel food
II Effect of the production process applied to the novel food
III History of the organism used as the source of the novel food
IX Anticipated intake/extent of use of the novel food
X Information from previous human exposure to novel food or its source
XI Nutritional information of the novel food
XII Microbiological information on the novel food
XIII Toxicological information on the novel food

Each section begins with a set of relevant questions taken from the decision tree for that particular section in document97/618/EC. Each question is answered as either yes or no and reference is given where, in the subsequent section evidence can be found to substantiate the answer.

I SPECIFICATION OF THE NOVEL FOOD

Application of the Decision Tree

Question	Answer	Comments
1. Is appropriate analytical information available on potentially toxic inherent constituents, external contaminants & nutrients?	Yes	Answers to these three questions are set out in sections I.1 through I.9
2. Is the information representative of the novel food when produced on a commercial scale?	Yes	
3. Is there an appropriate specification … to ensure that the novel food marketed is the same as that evaluated?	Yes	

I SPECIFICATION OF MODIFIED WHEAT STARCH

I.1 COMMON OR USUAL NAME

Modified Wheat Starch

I.2 CHEMICAL NAME

Phosphated Distarch Phosphate

I.3 TRADE NAMES

Fibersym® RW (formerly sold as Fibersym® 70) & FiberRite® RW

I.4 CHEMICAL ABSTRACT SERVICE (CAS) NUMBER

The CAS Number for phosphated distarch phosphate is 11120-02-8

I.5 CHEMICAL STRUCTURE

Wheat starch generally exists in two forms a linear helical molecule amylose and a multi-branched molecule amylopectin (discussed in detail in XI.2.1). Phosphorylation of the starch leads to the formation of cross links both within and between individual starch molecules.

I.6 MOLECULR FORMULA & WEIGHT

Phosphated distarch phosphate

$(C_6H_{10}O_5)_n\ [(C_6H_9O_5)_2PHO_2]_a[(C_6H_9O_5)PH_2O_3]_b$

I.7 CHEMICAL & PHYSICAL PROPERTIES

Modified Wheat Starch is a white or near white free flowing powder.

I.8 PRODUCT SPECIFICATION & SUPPORTING ANALYSES

I.8.1 European Purity Criteria for Phosphated Distarch Phosphate

Criteria for the purity of phosphated distarch phosphate have been set out in European Legislation (Directive 2000/63/EU; Commission of the European Communities, 2000) and are reproduced from that document in Table I-1 below:

Technical specifications and nutritional data for Fibersym® RW and FiberRite® RW as supplied to customers are summarised in Table I-2. Copies of the specifications supplied to customers are attached as Annex I-A.

Analytical quality control data supporting a number of the parameters covered by the specifications are summarised in Tables I-3 and I-4. In addition to these analyses, samples of raw material (wheat flour) are analysed in house or by external laboratories (Medallion Laboratories and Midwest Laboratories Inc.) annually to ensure that they meet heavy metal-, and pesticide- residue limits are complied with. These are summarised in Table I-5. Copies of relevant external laboratory reports are shown in Annex I-B.

I-9 SUMMARY

Through its programme of chemical and physical analyses it can be demonstrated that both Fibersym® RW and FiberRite® RW comply not only with relevant EU legislation but also with the company's own internal specifications.

Table I-1 Purity criteria for Phosphated Distarch Phosphate (E 1413) as set out in Commission Directive 2000/63/EU

Definition	Phosphated distarch phosphate is starch having undergone a combination of treatments as described for monostarch phosphate and for distarch phosphate.
Description	White or nearly white powder or granules or (if pregelatinised) flakes , amorphous powder or coarse particles
Identification	
A If not pregelatinised: by micro-scopic observation. B Iodine staining positive (dark blue to light red colour)	
Purity (all values expressed on an anhydrous basis except for loss on drying	
Loss on drying	Not more than 15.0% for cereal starch Not more than 21.0% for potato starch Not more than 18.0% for other starches
Residual phosphate	Not more than 0.5% (as P) for wheat or potato starch Not more than 0.4% (as P) for other starches
Sulphur dioxide	Not more than 50mg/kg for modified cereal starches Not more than 10mg/kg for modified starches, unless specified
Arsenic	Not more than 1 mg/kg
Lead	Not more than 2 mg/kg
Mercury	Not more than 0.1% mg/kg

Table I-2 Company specifications for two phosphated distarch phosphate products made from wheat starch

Analyte	Description		Method	Frequency
	Fibersym® RW	**FiberRite® RW**		
Physical				
Appearance	Fine Powder	Fine Powder	Visual	Every Lot
Colour	White to off white	White to off white	Visual	Every Lot
Odour	None	None	Sensory	Every Lot
Chemical				
Residual phosphorus	Not more than 0.4%	Not more than 0.4%	AOAC 995.1	Every lot
Arsenic	Not more than 1 mg kg^{-1}	Not more than 1 mg kg^{-1}	SW-8466010B R2.0	Annually
Lead	Not more than 2 mg kg^{-1}	Not more than 2 mg kg^{-1}	SW-8466010B R2.0	Annually
Mercury	Not more than 0.1 mg kg^{-1}	Not more than 0.1 mg kg^{-1}	SW-8467471A R1.0	Annually
PH (25% slurry)	4.5 – 6.5	4.5 – 6.5	PRL002 – pH meter	Every Lot
Ash	Not more than 3%		AACC 08-03	Every Lot
Nutritional data (g per 100g)l				
Moisture	10.6	12.5	PRL019 Mettler moisture meter	Every Batch
Energy (Calories)	356[1]	85[2]		
Total Dietary Fibre (dry matter basis)	76 (minimum)	65.6 (minimum)	AOAC 991.43	Every Batch
Ash	0.99	1.17	AACC 08-03	Nutritional Sample
Protein	0.5%	0.5%	LECO Combustion	Nutritional Sample
Total fat	0.50	0.34	GC	Nutritional Sample

Table I-2 Company specifications for two phosphated distarch phosphate products made from wheat starch

Analyte	Description		Method	Frequency
	Fibersym® RW	**FiberRite® RW**		
Microbiological				
Aerobic plate count	10,000 cfu[3]/g max	10,000 cfu/g max	FDA-BAM 8th Ed Rev.A Ch. 3	Every Lot
Moulds & Yeasts	200 cfu/g max	200 cfu/g max	FDA-BAM 8th Ed Rev.A Ch. 18	Every Lot
Escherichia coli	Negative	Negative	FDA-BAM 8th Ed Rev.A Ch. 4	Every Lot
Salmonella spp.	Negative	Negative	AOAC 990.13	Every Lot

Notes:
1 No fibre correction;
2 Corrected for insoluble dietary fibre;
3 cfu = colony forming units

Table I-3 Specimen internal quality control data for 5 lots of Fibersym® RW (previously known as Fibersym® 70)

Analyte	Specification	Lot Number				
		10-1000006-000056	10-100006-000116	10-100006-000010	10-100006-000126	10-100006-000174
% moisture	9 -12	10.02	9.7	9.99	9.98	10.18
pH (25% slurry)	4.5 – 6.5	6.0	6.0	6.0	5.9	5.9
Total Dietary Fibre (dry matter basis)	85%	95.6	99.0	97.4	91.8	97.5
Aerobic Plate Count	10,000 cfu/g (max)	110 cfu/g	10 cfu/g	40 cfu/g	10 cfu/g	10 cfu/g
Moulds & Yeasts	200 cfu/g (max)	< 10 cfu/g	< 10 cfu/g	< 10 cfu/g	< 10 cfu/g	< 10 cfu/g
Escherichia coli	Negative	Negative	Negative	Negative	Negative	Negative
Salmonella spp.	Negative	Negative	Negative	Negative	Negative	Negative

Table I-4 Specimen internal quality control data for 5 lots of FiberRite® RW

Analyte	Specification	Lot Number				
		10-1000020-000032	10-100020-000082	10-100020-000085	10-100020-000089	10-100020-00090
% moisture	9 - 12	11.49	9.8	10.8	10.0	9.8
pH (25% slurry)	4.5 – 6.5	6.0	5.5	5.6	5.5	5.5
Total Dietary Fibre (dry matter basis)	75% min	79.0%	86.0%	77.0%	84.0%	86.0%
Aerobic Plate Count	10,000 cfu/g (max)	1770 cfu/g	370 cfu/g	800 cfu/g	220	370
Moulds & Yeasts	200 cfu/g (max)	80 cfu/g	20 cfu/g	80 cfu/g	40 cfu/g	20 cfu/g
Escherichia coli	Negative	Negative	Negative	Negative	Negative	Negative
Salmonella spp.	Negative	Negative	Negative	Negative	Negative	Negative

Table I-5 Specimen raw material (wheat flour) analytical data

Analyte	Laboratory Name & Certificate Number	Result
Heavy Metals	Midwest laboratories Inc.	
Arsenic	08-184-2224	Not detected (<0.5 ppm)
Cadmium		Not detected (<0.05ppm)
Copper		1.63ppm
Lead		Not detected (<0.1ppm)
Mercury		Not detected (<0.05ppm)
Tin		Non detected (<1.0 ppm)
Zinc		8.36ppm
Pesticides	Medallion Laboratories	
Organo Halogens	2007-MED-4965	Not detected (<0.200mg/kg)
Organo Nitrogen		Not detected (< 0100mg/kg)
Organo Phosphates		Not detected (<0.050mg/kg)
N-methyl carbamates		Not detected (<0.100mg/kg)
Mycotoxins		
Deoxynivalenol	MGP Ingredients*	Not detected (<0.5 ppm)

* performed in house using the Neogen Verotox 5/5 rapid Test Kit (Method Approval GIPSA/FGIS 2002-106)

II EFFECT OF THE PRODUCTION PROCESS APPLIED TO THE NOVEL FOOD

Application of the Decision Tree

Question	Answer	Comments
1. Does the novel food undergo a production process	Yes	PDSP is produced using methods detailed within US, patents numbers 5 855 946 & 6 299 907
2. Is there a history of use of the production process for the food	Yes	MGP Ingredients has been manufacturing and selling PDSP using these patented methods under licence since February 2004.

II MANUFACTURE OF MODIFIED WHEAT STARCHES (FIBERSYM® RW AND FIBERRITE® RW)

II.1 UNIQUENESS OF PROCESS

The particular processes used to produce PDSP by MGP Ingredients are covered by Patent Numbers patents 5 855 946 & 6 299 907 (Seib & Woo, 1999, 2001). The company has been producing PDSP using this technology since February 2004.

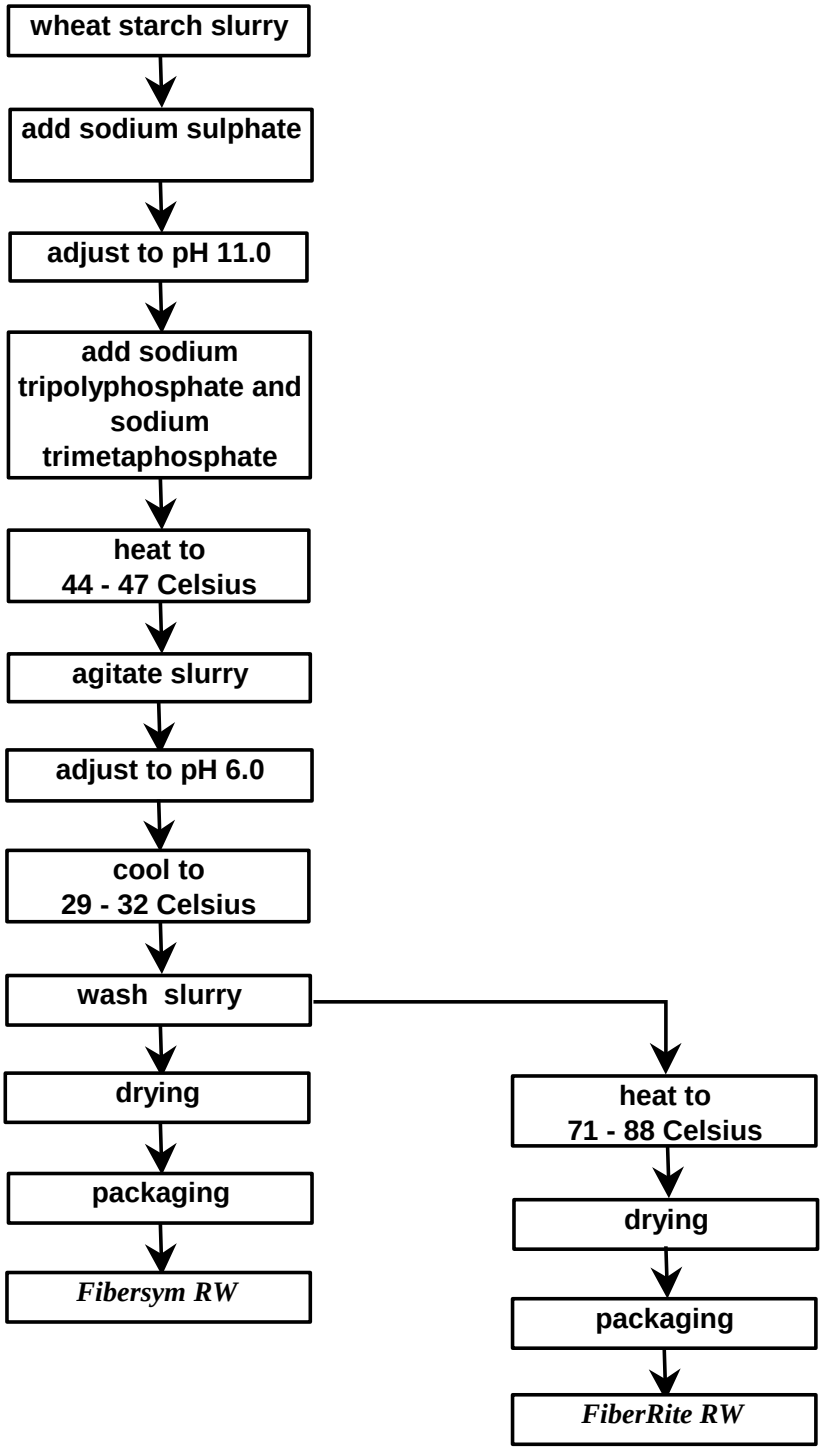
II.2 DETAILS OF PROCESS

The products are produced from wheat starch – this is usually manufactured by the applicant. The starch is esterified and cross-linked by treatment with sodium tripolyphosphate and sodium trimetaphosphate. When used individually, these chemicals are used in the production of monostarch- and distarch phosphates respectively (Commission of the European Communities, 2000).

The process used to produce the modified wheat starches FiberRite® RW and Fibersym® RW is shown in Figure II-1. Essentially a slurry of wheat starch is treated with phosphating agents under alkaline conditions at mildly elevated temperatures. The pH of the slurry is then brought to approximately pH 6.0 and then washed. The washed slurry is then either dried to produce Fibersym® RW or cooked at between 80 and 99 Celsius. The cooked slurry is then dried, packaged and sold as FiberRite® RW.

Products are subjected to batch by batch quality control analyses prior to sale as well as further periodic quality assurance tests. These have been detailed within section I.8 of this chapter.

Figure II-1 Process Flow Chart for Fibersym® RW and FiberRite® RW (In accordance with U.S. Patent 5,855,946)



II.3 PRODUCT SHELF LIFE

Both products have a shelf life of 12 calendar months

II.4 FOOD SAFETY MANAGEMENT SYSTEMS

Food safety management systems for the production of Fibersym® RW and FiberRite® RW operate under HACCP (**H**azard **A**nalysis **C**ritical **C**ontrol **P**oint) principles. Details of the studies underpinning operations are given in Annex II-A

II-4 SUMMARY

Fibersym® RW and FiberRite® RW are manufactured in accordance with previously published (and patented) methods with appropriate food safety management systems in place.

III HISTORY OF THE ORGANISM USED AS THE SOURCE OF THE NOVEL FOOD

Application of the Decision Tree

Question	Answer	Comments
1. Is the novel food obtained from a biological source, i.e. a plant animal or micro-organism	Yes	Starch is extracted from wheat
2. Has the organism used as the source of the novel food been derived using GM	No	No GM wheat is currently grown commercially
3. Is the source organism characterised	Yes	See 1 above
4. Is there information to show that the organism and/or foods obtained from it are not detrimental to human health	Yes	Wheat has a long history of use with the Member States.

III SOURCE ORGANISM

III.1 INFORMATION CONCERNING SOURCE ORGANISM

The source organism is wheat (*Triticum aestivi or T. durum*). As Kent and Evers (1994) have pointed out; wheat has been grown and eaten since antiquity. Archaeological studies have shown it being grown and consumed in Iran, Egypt, as well as Southern and Central Europe before the birth of Christ. Wheat and therefore wheat starch have a considerable history of use within Member States.

Historically wheat has been subjected to intensive breeding programmes both in member states and abroad. New varieties are subjected to an approval process before going into commercial production. Examples include that operated by the Home Grown Cereals Authority Recommended List (Home Grown Cereals Authority, 2007) in the United Kingdom and those in the United States which are regionally specific (e.g. Ross *et al* 2003).

Currently no GM-wheat is commercially grown in either North America, or the Member States of the European Union. Given current consumer attitudes, this is unlikely to change (Wisner, 2005).

III.2 SAFETY OF WHEAT STARCH

Wheat comprises approximately 62% starch (Kent and Evers, 1994). Given its history the cereal and its starch can be considered to be safe for consumption by most of the population. There are however two sets of the population for whom wheat is contra-indicated. These are those suffering from:

- Coeliac disease and related conditions which reflect a sensitivity to gluten leading, in the case of coeliac disease to degradation of the villi in the small intestine.
- True food allergy towards any one or more wheat proteins.

This question is addressed in subchapter XIII-3.

III.3 SUMMARY

FiberRite® RWand Fibersym® RW are produced from starch extracted from wheat a plant widely consumed for a considerable time and in various forms within the community.

IX ANTICIPATED INTAKE/EXTENT OF USE OF THE NOVEL FOOD

Application of the Decision Tree

Question	Answer	Comments
1. Is there information on the anticipated uses of the novel food based on its properties	Yes	Anticipated consumption can be derived by generation of models using data generated in the National Diet and Nutrition Surveys performed on various age groups within the UK., since 1989
2. Is there information to show anticipated intakes for groups predicted to be at risk	Yes	Analysis of the data generated through answering question 1 indicates that no population group is at risk
3. Will introduction of the novel food be restricted geographically	No	No geographical restrictions are anticipated.
4. Will the novel food replace other foods in the diet.	No	The food is intended as minor ingredient in compound foods – it is not intended to substantially replace any particular food or ingredient.

IX ANTICIPATED INTAKE OF FIBERSYM® RW & FIBERRITE® RW WITHIN THE EU

IX.1 PROPOSED LEVELS OF INCLUSION

Fibersym® RW and FiberRite® RW are intended for use as sources of additional dietary fibre in the form of type 4 resistant starch (see Section XI.2.3) in a range of diverse farinaceous foods including bread, pasta, morning goods and biscuits. Recipes for foods using flour replaced with phosphated distarch phosphate (PDSP) have been published (e.g. Maningat *et al.*, 2005, 2006; Dohl *et al.*, 2005). For the purposes of this application, approval is sought to include Fibersym® RW and FiberRite® RW into a range of foods at levels shown in Table IX-1.

Table IX-1 Proposed maximum levels of incorporation (g per 100g) of either of the MGP Ingredients Phosphated Distarch Phosphate (PDSP Products) Fibersym® RW and FiberRite® RW into a range of farinaceous products*

	White bread & related products	Processed ready to Eat breakfast Cereals	Pasta	Sweet Biscuits & Crackers	Cakes	Starch-Based Snack foods
PDSP	7.5	7.5	5	10	15	10

* *N.B. all data are expressed in terms of product as consumed*

IX.2 ESTIMATE OF ANTICIPATED INTAKE OF EITHER FIBERRITE® RW OR FIBERSYM® RW WHEN INCORPORATED INTO FARINACEOUS FOODS ON DIETARY FIBRE & DIETARY PHOSPHORUS CONSUMPTION

IX.2.1 Introduction

The estimated consumption of RS-4 and Phosphorus (assuming the inclusion of either Fibersym® RW or FiberRite® RW at the maximum levels in all of the foods for which permission is sought) was determined using individual food consumption data obtained from various United Kingdom National Diet and Nutrition Surveys (NDNS). These are Government funded surveys which constitute part of a rolling surveillance programme of both the nation's diet and its physiology. Surveys were originally commissioned by the UK Department of Health and the Ministry of Agriculture Fisheries & Foods. In 2000 responsibility for the surveys was transferred to the Food Standards Agency. Surveys are structured to take into account geographic and seasonal variation. Individuals and households surveyed are selected by means of a stratified multi-stage random probability design, with sampling being performed throughout the United Kingdom, based on postal codes. In addition, each survey is performed in 4 successive 'waves' over a 12 month period to take into account any seasonal variations in food consumption. All surveys were undertaken with the approval of the appropriate local ethical committees. Use has been made of the data from the last three surveys undertaken. These were:

- Adults aged between 16 and 64 years, collected between 2000 & 2001 (Henderson *et al*. 2002))
- Young people aged 4 to 18 years, collected in 1997 (Gregory *et al.*, 2000)
- Elderly persons (over 65 years), undertaken between 1994 and 1995 (Finch *et al.* 1998)

Collectively these surveys give the most detailed and up to date overview of the eating habits of the UK population. The survey data comprises individual 4- (adults aged 65 and over) or 7- day weighed-food records plus associated physiological and clinical data.. In each survey,

of those identified as suitable for participation, only a proportion (the respondents) went on to complete a full diet diary. These form the base line from which the analyses described in this section were determined. Individual food consumption information was generated by weighing foods before being eaten and recording the weight of anything left over after the meal had finished. Weighing was undertaken by the individuals themselves or, if incapable, their parents (children) or carers (elderly).

Details of each survey are detailed below.

National Diet and Nutrition Survey: People Aged 65 years and over, 1994-95 (Finch et al. 1998)

This survey was undertaken in a series of four three-month waves commencing October 1994 and finishing in September 1995. In addition to the age and geographic criteria discussed above; respondents were further selected on the basis of whether or not they lived in their own home or in an institution. 2172 home-dwelling and 452 institution dwelling individuals (total 2624) were identified as being eligible to participate in the survey. With regards to the keeping of the diet diary 59% of those living at home and 91% of those living in institutions completed a diary for the entire 4 days. In all 1687 individuals responded by submitting diet diaries for the entire 4 days.

National Diet and Nutrition Survey: Young People Aged 4 to 18 Years, 1997 (Gregory et al., 2000.)

The survey was undertaken in the calendar year 1997, with survey work beginning in January and finishing in December. The initial survey size comprised 2127 individuals aged between 4 and 18, of whom 1701 submitted a 7-day diet diary.

National Diet and Nutrition Survey: People Aged 19 to 64 (*Henderson et al., 2002)*

In common with other surveys, this one was undertaken in four three-month waves beginning in July 2000 and finishing in June 2001. In accordance with the criteria discussed above, 3704 individuals aged between 19 and 64 years old were identified as being eligible (eligibility required that respondents were neither pregnant nor breast-feeding). Of these 47% (1724 individuals) completed a 7-day diet diary to form the respondent-base on which analyses described here were performed.

For comparison purposes details of these three surveys are summarised in Table IX-2. In combination therefore, these data sets probably give the best estimate of current dietary habits within the United Kingdom and can be used as a reasonable basis to estimate of effects on EU consumption as a whole. The data sets were obtained under license from the UK Data Archive and the contents of the diet diaries from each survey analysed. Consumption data was used to estimate the daily amounts of RS-4 and phosphorus that might be consumed assuming that all of the food groups for which permission has been sought were supplemented with the maximum amount of either Fibersym® RW or FiberRite® RW applied for.

Table IX-2 Summary of UK National Diet & Nutrition Surveys, whose data was used in this evaluation

Population	When Surveyed	Number Invited	Diet Diaries Completed (%)	Reference
Young Persons (4 –18)	1997	2672	1701 (64)	Gregory *et al (2000)*
Adults (19 – 65)	2000/2001	2694	1724 (47)	Henderson *et al.* (2002)
Elderly (> 64)				
Living at home	1994/1995	2172	1275 (59)	Finch *et al.,* 1998
Living in care	1994/1995	454	412 (91)	

IX.2.2 Approach

Data from individual diet diaries were collated electronically. Using the known amounts of each food, covered by this application, consumed and the maximum permitted amounts of phosphated distarch phosphate applied for (see: Table IX-1); it was possible to derive estimates of consumption of RS-4 and additional phosphorus after proposed supplementation. Estimates of RS-4 and additional phosphorus consumption were derived either on a whole population ('respondent') or only on those who actually ate the product ('consumer') basis.

Consumption data are expressed on a daily basis, derived from the 7-day food-diaries kept by both young people and adults aged 19 to 64. In the case of people aged 65 and over, it should be noted that only 4-day diaries were kept. In estimating impacts on RS-4 and phosphorus consumption for this age-group, data was 'normalised' as described by Gregory (1995) with week day consumption being assumed to be similar over the 5 day period, weighted accordingly and combined with week-end data.

Consumption was estimated for the following populations:

- Male children aged 4 – 10 years
- Female children aged 4 – 10 years
- Male teenagers (aged 11 – 18 years)
- Female Teenagers (aged (11 – 18 years)
- Male Adults (aged 19 – 64 years)
- Female Adults (aged 19 –64 years)
- Male Elderly (aged 65 years and over)
- Female Elderly (aged 65 years and over).

IX.2.3 Results

Summary data for RS-4 consumption based on supplementation with either Fibersym® RW (Table IX-3) or FiberRite® RW (Table IX-4) for males and females of each age group are detailed below. Similar data for additional phosphorus consumption from either Fibersym® RW or FiberRite® RW are shown in Table IX-7. The latter data were calculated on the maximum residual bound phosphorus content of 0.4% which applies for either type of modified starch. Data are expressed in terms of estimated consumption (grams starch, milligrams phosphorus) on a per person per day basis. Details of RS-4 and phosphorus consumption attributable to the food groups evaluated for the purposes of these analyses are detailed in Annex IX-A. Total dietary exposure towards either RS-4 or additional phosphorus on a body weight basis are shown in Tables IX.-5 (RS-4 from Fibersym® RW), IX-6 (RS-4 from FiberRite® RW), and IX-8 (Phosphorus from either modified starch).

IX.2.3.1 *Estimated Overall Consumption of Modified Starches (Tables IX-3- IX-6)*

In terms of overall consumption, given the broad range of foods applied for permission to add the modified starches to, predicted consumption rates were high - often at or approaching 100%. Consequently discussion will be restricted to data calculated on a respondent basis.

Average predicted daily consumption of RS-4 was generally lower in females than males, the exception being male children who were expected to eat marginally less than their female counterparts (8.5g versus 9.9g for Fibersym® RW; 7.3g per person per day versus 8.5g for FiberRite® RW). Highest consumption was predicted for male teenagers. For Fibersym® RW, estimated average consumption was 15.2g per person per day, with intakes at the 90, 95 and 97.5 percentiles equivalent to 20.0, 32.8 and 48.7g per person per day respectively. For FiberRite® RW the estimated average intake was 13.1g per person per day, with intakes at the 90, 95 and 97.5 percentiles equivalent to 17.2, 28.3 and 42g per person per day respectively. In terms of their gender it would be predicted that female teenagers would also eat more than female children or adults (average intakes 10.4g and 9.0g and 10.4g RS-4 from Fibersym® RW and FiberRite® RW respectively (Tables 1 and 2). While highest RS-4 consumption was predicted in teenagers, the data suggests that RS-4 consumption would decrease through adulthood with average levels of intake in elderly females being lower than their child counterparts.

In terms of dietary impact and measured on a per kilogram body weight basis with regard to RS-4 intakes; consideration of Tables IX-5 and IX-6 suggests that the greatest impact would be on children. Highest consumption was estimated for female children (estimated mean respondent consumption of RS-4 from Fibersym® RW of 0.41g per kilogram bodyweight per day, and Fibersym® RW of 0.35g per kilogram body weight per day for FiberRite® RW). Lowest consumption was estimated in the elderly with elderly male respondents estimated to consume 0.08g RS4 per kg body weight per day.

Table IX-3 Estimated Overall Daily Consumption (g. per person per day) of RS-4 from Fibersym® RW by Age Group and Gender

Population Group	Age Group	n	*Respondents* Mean (g)	Percentile			% resp.	*Consumers* Mean (g)	Percentile		
				90	95	97.5			90	95	97.5
Male Children	4 - 10	418	8.5	13.2	13.4	13.7	100	8.5	13.2	13.4	13.7
Female Children	4 - 10	431	9.9	13.6	14.5	15.6	100	9.9	13.6	14.5	15.6
Male Teenagers	11-18	416	15.2	20.0	32.8	48.7	100	15.2	20.0	32.8	48.7
Female Teenagers	11 – 18	436	10.4	15.3	16.4	18.4	100	10.4	15.3	16.4	18.4
Male Adults	19 – 64	893	9.9	15.3	18.5	20.6	98.1	10.0	15.3	18.6	20.8
Female Adults	19 – 64	722	7.6	13.0	14.3	16.7	100	7.6	13.0	14.3	16.7
Male Elderly	≥ 65	834	8.5	14.1	15.7	16.9	100	8.5	14.1	15.7	16.9
Female Elderly	≥ 65	851	7.1	11.8	14.1	14.3	98.0	7.0	11.8	14.1	14.3

Table IX-4 Estimated Overall Daily Consumption (g per person per day) of RS-4 from FiberRite® RW by Age Group and Gender

Population Group	Age Group	n	*Respondents* Mean (g)	Percentile			% resp.	*Consumers* Mean (mg)	Percentile		
				90	95	97.5			90	95	97.5
Male Children	4 - 10	418	7.3	11.4	11.5	11.8	100	7.3	11.4	11.5	11.8
Female Children	4 - 10	431	8.5	11.8	12.5	13.4	100	8.5	11.8	12.5	13.4
Male Teenagers	11-18	416	13.1	17.2	28.3	42.0	100	13.1	17.2	28.3	42.0
Female Teenagers	11 – 18	436	9.0	13.2	14.2	15.9	100	9.0	13.2	14.2	15.9
Male Adults	19 – 64	893	9.1	14.1	17.0	19.0	98.1	9.3	14.1	17.2	19.1
Female Adults	19 – 64	722	7.0	11.9	13.2	15.4	100	7.0	11.9	13.2	15.4
Male Elderly	≥ 65	834	8.5	14.1	15.7	16.9	100	8.5	14.1	15.7	16.9
Female Elderly	≥ 65	851	7.1	11.8	14.1	14.3	98.0	7.0	11.8	14.1	14.3

Table IX-5 Estimated Overall Daily Consumption of RS-4 (g per kg body weight per day) from Fibersym® RW by Age Group and Gender

Population Group	Age Group	*Respondents* n	Mean (g)	Percentile 90	95	97.5	% resp.	*Consumers* Mean (g)	Percentile 90	95	97.5
Male Children	4 - 10	418	0.34	0.50	0.51	0.52	100	0.34	0.50	0.51	0.52
Female Children	4 - 10	431	0.41	0.58	0.59	0.75	100	0.41	0.58	0.59	0.75
Male Teenagers	11-18	416	0.24	0.36	0.44	0.51	100	0.24	0.36	0.44	0.51
Female Teenagers	11 – 18	436	0.21	0.31	0.33	0.34	100	0.21	0.31	0.33	0.34
Male Adults	19 – 64	893	0.12	0.20	0.25	0.29	98.1	0.13	0.21	0.26	0.29
Female Adults	19 – 64	722	0.10	0.18	0.22	0.25	100	0.10	0.18	0.22	0.25
Male Elderly	≥ 65	834	0.08	0.12	0.14	0.14	100	0.08	0.12	0.14	0.14
Female Elderly	≥ 65	851	0.08	0.15	0.22	0.23	98.0	0.08	0.15	0.22	0.23

Table IX-6 Estimated Overall Daily Consumption of RS-4 (g per kg body weight per day) from FiberRite® RW by Age Group and Gender

Population Group	Age Group	*Respondents* n	Mean (g)	Percentile 90	95	97.5	% resp.	*Consumers* Mean (mg)	Percentile 90	95	97.5
Male Children	4 - 10	418	0.29	0.43	0.44	0.45	100	0.29	0.43	0.44	0.45
Female Children	4 - 10	431	0.35	0.50	0.51	0.65	100	0.35	0.50	0.51	0.65
Male Teenagers	11-18	416	0.20	0.31	0.38	0.44	100	0.20	0.31	0.38	0.44
Female Teenagers	11 – 18	436	0.20	0.31	0.38	0.44	100	0.20	0.31	0.38	0.44
Male Adults	19 – 64	893	0.11	0.18	0.22	0.25	98.1	0.11	0.18	0.22	0.25
Female Adults	19 – 64	722	0.09	0.15	0.19	0.22	100	0.09	0.15	0.19	0.22
Male Elderly	≥ 65	834	0.07	0.11	0.12	0.12	100	0.07	0.11	0.12	0.12
Female Elderly	≥ 65	851	0.07	0.13	0.19	0.20	98.0	0.07	0.13	0.19	0.20

IX.2.4.2 *Estimated Overall Consumption of Phosphorus (Tables IX-7 & IX-8)*

Predicted additional phosphorus consumption is detailed in Table IX-7. In terms of its predicted consumption, the relative amounts consumed by the different populations reflected

those obtained for RS-4. Consequently the highest intake of additional phosphorus was seen in male teenagers (average 80mg per day with intakes at the 90, 95 and 97.5 percentiles equivalent to respectively 105, 173 and 256mg per person per day). Similar predictions made for other population groups described above for RS-4 also apply in the case of phosphorus.

In terms of dietary impact and measured on a per kilogram body weight basis (Table IX-8); as in the case of RS-4, highest consumption of phosphorus was estimated for female children (estimated mean respondent consumption of phosphorus 2.14mg phosphorus per kg body weight per day). Lowest consumption was estimated in the elderly, with elderly male respondents, who were estimated to consume 0.42mg per kg body weight per day.

Table IX-7 Estimated Overall Daily Consumption (mg per person per day) of Additional Phosphorus from either Fibersym® RW or FiberRite® RW by Age Group and Gender

Population Group	Age Group	n	*Respondents*				% resp.	*Consumers*			
			Mean (mg)	Percentile				Mean (mg)	Percentile		
				90	95	97.5			90	95	97.5
Male Children	4 - 10	418	45	69	70	72	100	45	69	70	72
Female Children	4 - 10	431	52	72	76	82	100	52	72	76	82
Male Teenagers	11-18	416	80	105	173	256	100	80	105	173	256
Female Teenagers	11 – 18	436	55	80	86	97	100	55	80	86	97
Male Adults	19 – 64	893	52	81	97	108	98.1	53	81	98	110
Female Adults	19 – 64	722	40	68	75	88	100	40	68	75	88
Male Elderly	≥ 65	834	52	86	96	103	100	52	86	96	103
Female Elderly	≥ 65	851	44	72	86	87	98.0	43	72	86	87

Table IX-8 Estimated Overall Daily Consumption of Additional Phosphorus (mg per kg body weight per day) from either Fibersym® RW or FiberRite® RW by Age Group and Gender

Population Group	Age Group	n	*Respondents* Mean (mg)	*Respondents* Percentile 90	95	97.5	% resp.	*Consumers* Mean (mg)	*Consumers* Percentile 90	95	97.5
Male Children	4 - 10	418	1.78	2.62	2.68	2.72	100	1.78	2.62	2.68	2.72
Female Children	4 - 10	431	2.14	3.04	3.10	3.93	100	2.14	3.04	3.10	3.93
Male Teenagers	11-18	416	1.25	1.92	2.29	2.68	100	1.25	1.92	2.29	2.68
Female Teenagers	11 – 18	436	1.08	1.63	1.72	1.79	100	1.08	1.63	1.72	1.79
Male Adults	19 – 64	893	0.66	1.07	1.34	1.54	98.1	0.67	1.08	1.36	1.55
Female Adults	19 – 64	722	0.55	0.94	1.15	1.32	100	0.55	0.94	1.15	1.32
Male Elderly	≥ 65	834	0.42	0.64	0.74	0.75	100	0.43	0.65	0.74	0.75
Female Elderly	≥ 65	851	0.44	0.78	1.18	1.21	98.0	0.44	0.78	1.18	1.21

IX.2.4.3 *Estimated Daily RS-4 and phosphorus intake from Individual Food Uses in the EU*

Analyses on the basis of individual foods and population group are detailed in tables forming part of Annex IX-A. Irrespective of population group the major source of RS-4 in terms of average consumption was white bread and associated products. The lowest values were predicted for elderly females (Fibersym® RW: 3.5g per day, 90, 95 and 97.5 percentiles equivalent to 7.4, 9.1 and 10.7g per day; FiberRite,® RW 3.0g per day, 90, 95 and 97.5 percentiles equivalent to 6.3, 7.8 and 9.3g per day;). The highest values were calculated for adult males (Fibersym® RW 6.4g per day, 90, 95 and 97.5 percentiles equivalent to 11.3, 12.2 and 12.4g per day; FiberRite® RW 5.9g per day, 90, 95 and 97.5 percentiles equivalent to 10.4, 11.2 and 11.4g per day;).

IX.3 PROPOSED GEOGRAPHIC DISTRIBUTION OF FIBERRITE® RW AND FIBERSYM® RW PRODUCTS WITHIN THE EUROPEAN COMMUNITY.

The proposed distribution of the two modified starches will be throughout the European Community depending on prevalent market conditions.

IX-4 SUMMARY

Estimated consumption data has been generated using the most up to date diet-diary based information available for the United Kingdom.. In terms of population (approximately 61 million) the United Kingdom represents a significant sample of the overall EU population (497 million, Eurostat, 2008). The calculations assume an extreme case; i.e. that all foods subject to this application would be supplemented with either modified wheat starch at the maximum amount for which permission is sought – the most unlikely scenario that could be considered. Under these circumstances, since RS-4 can be considered to be dietary fibre (discussed in XI.2.4.3), supplementation could be seen to have a positive effect on the dietary fibre consumption of the population.

X INFORMATION FROM PREVIOUS HUMAN EXPOSURE TO THE NOVEL FOOD OR ITS SOURCE

Application of the Decision Tree

Question	Answer	Comments
1. Is there information from previous direct , indirect, intended or unintended exposure to the novel food or its source which is relevant to the Community situation with respect to population, preparation, population. lifestyles and intakes	Yes	Phosphated distarch phosphate has a long history of use within the Community as a food additive. Modified wheat starch is permitted for use in other countries including the United States and Chile. Wheat is a key staple in the Community's diet.
2. Is there information to demonstrate that exposure to the NF is unlikely to give rise to nutritional, microbiological, toxicological and/or allergenicity problems	Yes	Not only has phosphated distarch phosphate enjoyed a long history of use as a food additive within the community, it has also been subjected to considerable toxicological evaluation as discussed in addressing questions under point XII

X CURRENT AND PREVIOUS USE OF MODIFIED WHEAT STARCH WITHIN MEMBER STATES AND THE REST OF THE WORLD

X.1 CONSUMPTION WITHIN THE EUROPEAN UNION

Modified wheat starch as phosphated distarch phosphate is a permitted food additive within the European Community (E 1413). Under current regulations (European Parliament and Council Directive 95/2/EC, as amended), its inclusion is generally permitted in foods at '*quantum satis'* levels. By definition, modified wheat starch is not a natural component of the diet. Consideration of recent DEFRA (Department of Environment, Food and Rural Affairs) data indicates that average starch consumption within the UK is approximately 156g per person per day, representing 26.4% of the total average daily dietary energy intake.

In terms of phosphated distarch phosphate no data is available as to how it is currently consumed within the European Union. FASEB has advised that for the purposes of food safety evaluations it assumes an intake of 17g per day of all modified starches (FASEB, 1979).

X.2 CONSUMPTION ELSEWHERE

Modified wheat starch is being used elsewhere in the world, in particular the Americas. Details of example products are given in Table X-1. Products include bread (including bread

directed at the child and young persons market, morning goods, biscuits, tortillas, pasta and ready meals).

Table X-1 Examples of Products Containing Either Fibersym® RW or FiberRite® RW on Sale in the Americas and Australia.

Manufacturer	Brand	Product	Description of PDSP Used	Country of Sale
[redacted]	[redacted]	White Bread	Almidón de Trigo	Chile
[redacted]	[redacted]	Various tortilla based ready meals	Modified Wheat Starch	United States of America
[redacted]		Flour Tortillas	Modified Wheat Starch	United States of America
[redacted]		Malt Biscuits Rich Tea Biscuits	Modified Starch	Australia
[redacted]	[redacted]	Biscuits (various recipes)	Modified Wheat Starch	United States of America
[redacted]	[redacted]	Bread	Modified Wheat Starch	United States of America
[redacted]	[redacted]	Muffin	Modified Food Starch	United States of America
[redacted]		Thin spaghetti	Modified Wheat Starch	United States of America

X.3 GENERAL FOOD SAFETY OF MODIFIED WHEAT STARCH

X.3.1 Microbiological Safety

Fibersym® RW and FiberRite® RW are manufactured to strict microbiological standards (see I.8) and are not considered to present any more significant microbiological hazard compared to unmodified wheat starch.

X.3.2 Toxicological Issues

The state of knowledge concerning the toxicology of phosphated distarch phosphate is detailed in the section XIII.2 below.

X.3.3. Food Allergy Issues

The question of any food allergy implications is addressed under XIII.5

XI NUTRITIONAL INFORMATION ON THE NOVEL FOOD

Application of Decision Tree

Question	Answer	Comments
1. Is there information to show that the novel food is nutritionally equivalent to existing foods that it might replace in the diet?	Yes	PDSP is a type 4 resistant starch (Section XI.2). Its starch hydrolysis & *in vitro* fermentability properties (Section XI.3) compare with published results obtained for other resistant starches (hydrolysis) or its parent starch, (fermentability). Furthermore when included in cooked foods (muffins or cereal bars), its effects on post-prandial glycaemia and insulinaemia are comparable with products containing other types of resistant starch (Section XI.4).

XI NUTRITIONAL INFORMATION ON PHOSPHATED DISTARCH PHOSPHATE (PDSP)

XI.1 INTRODUCTION

PDSP is considered to be a resistant starch; that is a form of starch, which is partially or completely resistant to amylolytic breakdown within the small intestine. In order to discuss the nutritional equivalence of PDSP it is necessary to discuss its performance within the context of current knowledge concerning starch chemistry (particularly as it applies to food processing) and the digestion of starch within the alimentary tract.

XI.2 STARCH CHEMISTRY & METABOLISM

X1.2.1 Molecular Structure and Organisation

Guy (2006) has recently reviewed the chemistry and food applications of starch. Starch is a botanical polysaccharide consisting of glucose monomers. It is found primarily in seeds and tubers, where it acts as the principle store of carbohydrate. In its natural state, starch is stored in discreet physical structures called starch granules. These granules have ordered structures within them that can be shown to have birefringence properties when examined under a microscope. With regards to its chemical structure; the glucose molecules within starch are linked by α-(1 → 4) bonds together with some branch points along the chain formed by α-(1 → 6) bonds. Starch exists in two distinct molecular forms:

Amylose – consisting of long linear polymers with 99% α-(1 → 4) bonds and very few α-(1 → 6) linkages. Chain lengths generally vary from 200 to 2000 glucose units. The nature of

the bond angles between the glucose residues permits the molecule to assume an alpha-helical structure.

Amylopectin – is the name given to a family of larger molecules with complex branched structures. These structures consist of about 95% α-(1 → 4) bonds and 5% α-(1 → 6) linkages. The presence of these linkages leads to the formation of highly branched structures consisting of both secondary and tertiary branches. Amylopectin molecules may contain up to 2 million glucose residues. Chemical structures of amylose and amylopectin are shown in Figure XI-1.

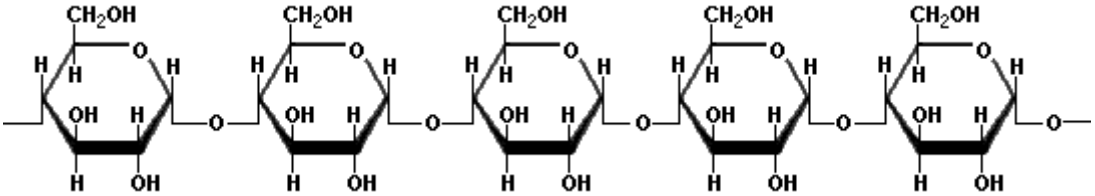
The proportions of amylose and amylopectin present within a starch granule are genetically determined. This fact has been utilised in conventional plant breeding programmes to produce varieties of cereals either high in amylopectin (so called 'waxy' varieties) or amylose. Within the starch granule, substantial amounts of amylopectin exist in ordered crystalline structures depending on a number of factors. These include molecular chain length, degree of packing and the presence of water (Katz, 1934; Wu and Sarko, $1978_{a,b}$: Gidley, 1987). The resultant granule structures are defined as, Type A –(usually found in cereals); Type B (mainly found in raw potato and banana) and; Type C (typically found in legumes).

XI.2.2 Effects of Food Processing on Starch Structure & Digestibility

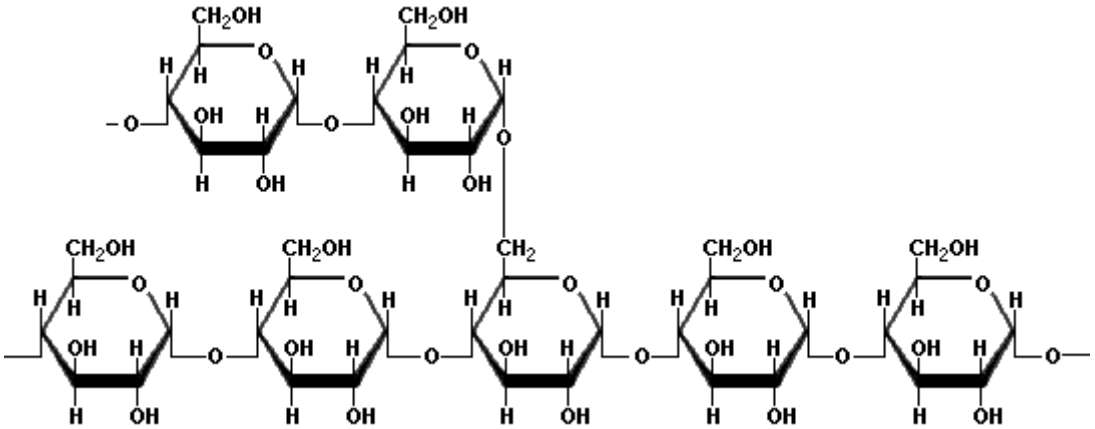
When heated in the presence of water, starch granules swell and eventually lose their birefringence properties. This process is termed gelatinisation. Loss of birefringence occurs at a specific temperature (T_m), depending on the botanical source of the starch and the amount of water used in cooking. The phenomenon represents a melting-like process where the crystalline structure of the starch within the granule is lost. Under suitable conditions of moisture and temperature, the starch granule will continue to swell and the starch assumes an amorphous structure (Guy, 2006). Starch polymers are unstable in solution and have a tendency to form complexes with each other and recrystalise on cooling - a process generically referred to as retrogradation. Depending on the relative proportions of amylose and amylopectin this can lead to the formation of gels or vitreous matter.

Processing also affects starch digestibility. Studies with canulated pigs (Wunsche, *et al.*, 1987) have shown that approximately 24% of potato starch from rations containing raw potatoes, escapes digestion in the small intestine. When pigs were fed freshly-cooked potato the proportion of starch escaping digestion was less. The consequences of cooking on starch digestibility in humans have also been studied. For example, Englyst and Cummings (1987), looked at the digestion of potato starch in ileostomy patients. In one study, subjects were maintained on a plant-polysaccharide free diet for 24 hours and then given one of two test meals, each containing 300g of potato cooked in a different way. The potato was either freshly cooked or; cooked and allowed to cool. Only 3% of the total starch consumed was recovered from subjects fed the freshly cooked potato. This increased to 12% when the potato was allowed to cool before being eaten. In a second study reported in the same paper; subjects were again maintained on a plant-polysaccharide free diet for 24 hours and then fed meals containing either cooled or reheated cooked potato. In this case approximately 13% of the starch eaten was recovered from ileostomy patients fed the cooled potato meal and about 7.5% from the meal containing reheated potato.

Figure XI-1 Chemical structures of amylose and amylopectin



amylose



amylopectin

In terms of its ability to provide sustenance, both the food matrix and the physico-chemical structure of the starch are extremely important in terms of starch digestibility. With regard to matrix effects, Heaton *et al* (1988) demonstrated that the insulin responses to whole or coarsely ground grains were less than those seen for finely ground flour from the same botanical source. The degree of mastication is also important. For example after meals of sweet corn, peas or beans, up to 20% of faecal solids might be starch in undigested food matter; the quantity of starch being determined by the degree to which the food was originally chewed (Englyst, 1985). In terms of physico-chemical structure; raw amylopectin appears to be more readily digested than raw amylose. This has been demonstrated in both rodents (Kabir *et al.* 1998) and ileostomy subjects (Muir *et al*., 1995).

In summary, during food processing, starch granules are gelatinised and are consequently more easily digested within the small intestine. Furthermore, even when processed, amylopectin is more susceptible than amylose to amylolytic attack. However, once cooled, starch may form new physical structures, less susceptible to amylolytic attack than the parent starch in its original botanical form (British Nutrition Foundation, 1990).

XI.2.3 The Concept of Resistant Starch

The observations that both raw and cooked forms of starch were not completely digestible by α-amylases led to the concept of resistant starch (Berry, 1986). In other words, starch which escaped exhaustive treatment with intestinal α-amylases *in vitro*. The concept was taken

further by Englyst and Cummings (1987) who classified starch into three types based on their ease of digestion within the small intestine. These were:

Rapidly digestible starch (RDS) – this comprises mainly of amorphous and well-dispersed starch such as that found in products cooked with significant quantities of water such as bread and cooked potato products.

Slowly digestible starch (SDS) – this class of starch is expected to be completely digested within the small intestine, albeit at a slower rate than RDS. It consists principally of amorphous starch, which is rendered poorly accessible to amylolytic enzymes due to physical constraints as well as raw starches in type A or type C granule forms.

Resistant starch (RS) – this class of starch is to one degree or another resistant to the effects of enzymic breakdown within the small intestine and consequently passes into the large intestine, where it might be fermented by the bowel microflora. Resistant starch can be subdivided into four groups (Englyst & Cummings, 1987; Baghurst *et al.*, 1996). These are:

RS_1 Starch resistant by virtue of its physical inaccessibility (e.g. coarsely milled grains)
RS_2 Starch present in a granular form, which is particularly resistant to digestion (e.g. as found in potatoes, bananas and amylose-rich starch granules)
RS_3 This is retrograded material formed during the cooling of gelatinised (cooked) starch and which has acquired a glass-like structure
RS_4 Chemically modified starches that may or may not be fermented in the colon.

The health properties of resistant starch were recently reviewed by Nugent (2005). As will be discussed further, the major benefits accruing to resistant starch probably relate more to its effects on the large bowel, rather than the entero-insular axis (Topping and Clifton, 2001, and discussed further in section XI.2.4.1 and XI.2.4.2). Essentially, resistant starch acts as a food for the indigenous colonic microflora. As such, its utilisation leads to the production of beneficial secondary metabolites, principally short chain fatty acids (SCFA). The two key health-promoting properties of these effects are provision of a preferred energy source (butyrate) for the colonic epithelia. This is considered to lead to beneficial trophic effects and a lowering of the pH of the colonic environment. The lowering in pH has a prebiotic effect, giving protection against colonisation by pathogenic bacteria and favouring the growth of 'beneficial bacteria (e.g. *Lactobacillus* spp. and *Bifidobacterium* spp.). The consequential changes in the bacterial microflora also contribute to a suppression of bacterial metabolism of primary bile acids to the potentially carcinogenic secondary forms.

XI.2.4 Nutritional Attributes of Resistant Starch

XI.2.4.1 *Physiological Aspects of Resistant Starch: Starch Digestibility and Blood Glucose Control*

Given that resistant starch was/is defined in terms of its resistance to amylolytic enzymes in the gut; it is no surprise that much attention has focussed on its ability or otherwise to modulate both the glycaemic response and the activity of the entero-insular axis. A key factor to be borne in mind is that the ability of resistant starches to withstand amylolysis reduces when they are included into processed foods. This was demonstrated by Brown *et al.* (2003), who studied the effects of meals containing varying amounts of either raw or cooked amylose

on post-prandial insulinaemia. This group fed rats meals containing increasing proportions of either raw or cooked amylose (15%, 34% or 48% dietary energy). The meals were both isocaloric and contributed the same amount of starch as energy (57%). Postprandial glycaemia and insulinaemia were studied for the following four hours. No meal related differences in glycaemia were observed. In the case of insulinaemia for rats fed the raw starch rations, reductions (both in terms of peak response and area under the curve) were seen; the degree of reduction being proportional to the amount of amylose included in the rat rations. Although these changes in insulinaemia were observed in rats fed cooked amylase, the amount of amylose included in the rations and needed to achieve the effect was higher

With regards to humans, in her review, Nugent (2005) observed that fifteen studies had reported an improvement in post prandial glycaemia and/or insulinaemia associated with resistant starch, while ten studies reported either no or physiologically insignificant effects. She concluded that while RS was associated with small reductions in glycaemia; its most potent effects were with regard to the attenuation of postprandial insulinaemia. Reflecting the findings of Brown *et al.* (2003), these effects also appear to be mediated by the food matrix within which the resistant starch is incorporated. Consideration of the literature concerning amylose (RS_2) highlights this. Work by Grandfeldt *et al* (1995) demonstrated that subjects eating corn bread made with high amylose corn starch gave blunter blood -insulin and –glucose responses than those made with standard (high amylopectin) maize flour. However in this case it should be noted that the products went through a cooling and reheating cycle, leading to the additional generation of retrograded starch (RS_3).

Behall's group (Behall *et al.*, 1988, Behall & Hallfrisch, 2002) studying the effects of amylose on postprandial blood glucose and insulin levels reported that when incorporated at 70% into crackers, amylose brought about a bluntening of the insulinaemic response, although no effect on glycaemia was observed. In a subsequent study, the same group administered bread containing increasing amounts of amylose to a group of non diabetic, but in some cases over-weight, subjects. They observed that effects on postprandial –glycaemia and -insulinaemia only occurred when the resistant starch was included at high levels (14.4 & 16.7%) within the product. Further work (Behall, *et al*. 2006) looked at the effects of feeding muffins with different resistant starch (amylose) and β-glucan contents in normal and over weight individuals. They found that in both cohorts, when fed high amylose muffins in a low β-glucan background, although no effect on postprandial glycaemia was seen, there was a bluntening effect on insulinaemia.

The ability of RS to attenuate post-prandial glucose uptake and insulin production in Type II diabetics has been mixed and again appears to reflect not only its source but also the quantity consumed. Coulston *et al* (1984) studied the effects of different starch sources (potato, rice, spaghetti, and lentils) on postprandial glycaemia and endocrine responses in non insulin dependent diabetes mellitus (NIDDM) patients and saw no differences in responses for any of the parameters studied. Investigations concerning the relationship between preparation (degree of boiling) and amylose content of rice (Larsen *et al*., 1996) in NIDDM patients indicated that that the increased amylose contents of rice were associated with lower GI values.

XI.2.4.2 *Physiological Aspects of Resistant Starch: Colonic Function*

One of the earliest roles assigned to resistant starch in humans was as a mitigating factor in the development of diverticular disease. Breath hydrogen studies by Thornton *et al.* (1986) in diverticular patients compared with age and sex matched healthy controls demonstrated that less starch from a potato meal entered the colons of the diverticular patients compared with the healthy subject cohort. From these results the authors hypothesised that the bacterial metabolites of undigested starch within the colon might have a protective effect.

The major bacterial metabolites of resistant starch within the colon are considered to be typical fermentation products (carbon dioxide, methane, hydrogen, organic acids (e.g. lactic acid) and short chain fatty acids (SCFA) such as acetic- butyric- and propionic- acids). The overall reaction from hexose (e.g. glucose produced by bacterially mediated amylolysis) has been described by Cummings (1997) as:

$$59C_6H_{12}O_6 + 38H_2O \rightarrow 60CH_3COOH + 22CH_3CH_2COOH + 18CH_3(CH_2)_2COOH + 96CO_2 + 268H^+ + \text{heat} + \text{additional bacteria}$$

While this can be considered a generic formula, it should be borne in mind that different resistant starches can behave differently in different environments. Martin *et al* (2000) compared the consequences of isocaloric diets high in either potato starch (RS_2) or retrograded amylose (RS_3) on the presence of SCFA in the portal blood supply of pigs. The total SCFA concentration within the hepatic portal vein of pigs fed potato starch was significantly ($P < 0.001$) less than that found in the retrograded amylose fed animals (305.4 versus 363 μmoles per litre). Not only were absolute quantities of SCFA absorbed into the venous blood supply different but so too were the molar ratios of the SCFA obtained (potato starch - 0.77:0.16:0.07 (acetate:propionate:butyrate); retrograded amylose - 0.89:0.10:0.01). Furthermore, in performing such experiments it is necessary to realise that the colonic microflora often needs time to adapt to be able to metabolise resistant starches. For example Henningsson *et al* (2003) fed Wistar rats isocaloric diets containing either raw potato starch or high amylose starch. They observed that the caecal SCFA pool not only changed as the study progressed (both in terms of the total amount of SCFA and relative amounts of different SCFA's), but that these changes were diet dependent. Thus by the end of the trial the caecal pool SCFA content of rats fed raw potato starch rose from 295μmol (SCFA molar ratio acetic:proprionic:butyric – 0.80:0.14:0.06) at day 13 of the trial to 1 380μmol (SCFA molar ratio: 0.73:0.11:0.16) at day 42. This compared with 163.6μmol (SCFA molar ratio 0.83:0.15:0.04) at day 13 rising to 1 013μmol (SCFA molar ratio 0.76:0.16:0.08) at day 42 for rats fed a high amylose starch diet.

Prior to the work of Thornton *et al.* (1986), Roediger (1980_a) had shown that butyrate, as produced by the endogenous colonic microflora was the preferred fuel of colonocytes. He went on to propose (Roediger, 1980_b), that diseases such as ulcerative colitis were in fact, manifestations of an inadequate energy supply (in the form of butyrate). Subsequent work has shown that butyrate has a protective effect against malignant transformation and promotes growth-arrest, differentiation and apoptosis in tumour cell lines (Reviewed in Topping and Clifton, 2001). Butyrate can also protect against both genotoxin (azoxymethane) insult in the colon (e.g. Leu *et al,* 2003) as well as chemically induced ulcerative colitis in the rat (Morita *et al.,* 2004; Moreau *et al.,* 2004). Therefore with regards to its contribution to bowel health, resistant starch, makes not only a substantial contribution to the energy requirements of the

large bowel microflora (Topping & Clifton, 2001; Topping *et al.*, 2003) but also has indirect (e.g. through SCFA) and beneficial trophic effects on the colon (Topping and Clifton, 2001).

XI.2.4.3 *Resistant Starch and Dietary Fibre*

Resistant starch is considered by some to be a type of dietary fibre (DeVries, 2003).

Current European Union (European Commission, 2008) legislation states that

For the purposes of this Directive "fibre" means carbohydrate polymers with three or more monomeric units, which are neither digested nor absorbed in the human small intestine and belong to the following categories:

— *edible carbohydrate polymers naturally occurring in the food as consumed;*

— *edible carbohydrate polymers which have been obtained from food raw material by physical, enzymatic or chemical means and which have a beneficial physiological effect demonstrated by generally accepted scientific evidence;*

— *edible synthetic carbohydrate polymers which have a beneficial physiological effect demonstrated by generally accepted scientific evidence*

XI.3 THE RESISTANT STARCH PROPERTIES OF PHOSPATED DISTARCH PHOSPHATE (PDSP) & OTHER TYPE RS_4 STARCHES

XI.3.1 Susceptibility to enzymic hydrolysis

In vitro experiments simulating starch hydrolysis, using either porcine pancreatic α-amylase (Kohn & Kay, 1963_a) or a mixture of porcine pancreatin and small intestinal mucosal preparations (Leegwater, 1971) have been performed. Both studies indicated that PDSP was more resistant to amylolysis than native starch.

More recently, studies by Seib's group have investigated the susceptibility of DPSP (produced by same technology as that used by the applicant). Initial studies on PDSP made from wheat starch (Woo and Seib, 2002) showed that the relative ratios of rapidly digestible-, slowly digestible- and resistant– starch (*see XI.2.3 for definitions*), as measured by the method of Englyst *et al* (1992) changed with increasing levels of phosphorylation. Increased phosphorylation led to increased amounts of both slowly digestible and resistant starch. However the ratios of these proportions differed depending on whether or not the starch had been pre-gelatinised immediately prior to testing. Pre-gelatinisation of starch led to increased susceptibility of the substrate to amylolysis. This manifested itself primarily in substantially increased amounts of rapidly digestible starch compared with the 'raw' equivalent. As in the case of the raw starch, the proportion of resistant starch increased as a function of the degree of phosphorylation. Subsequent work by this group (Shin *et al.*, 2004), demonstrated that this effect was not unique to phosphorylated wheat starches but also to phosphorylated derivatives of starches from other botanical sources (maize, rice and potato) as well. In the same paper they also demonstrated that the ability of phosphorylation to modify sensitivity to α-amylase was dependent on the botanical source of the starch. Thus in terms of total dietary fibre measurements, phosphorylated potato starch was the most resistant followed, in descending order, by that derived from wheat, maize and rice.

The observation that thermal processing may alter the sensitivity of PDSP to amylolytic attack is not unique to it, or, for that matter, RS_4 starches. Lee *et al*. (1985) demonstrated that the susceptibility of a number of RS_2 (tapioca, and potato starches) and RS_4 starches (tapioca

distarch phosphate, waxy maize distarch phosphate, acetylated waxy maize distarch phosphate and acetylated waxy maize distarch adipate) to pancreatic amylase increased when the starch was thermally processed.

The mechanism by which phosphorylation inhibits sensitivity to amylolytic attack is believed to related to the mechanism by which α-amylase attacks starch within the granule. Work by Gallant *et al.* (1972, 1997) has shown that starch in granules from cereals and cassava is digested from the inside out, while starch in potato granules is eroded from the surface. Cross-linking of starch causes obstructions in the porous channels leading into the interior of the granule and thereby blocks access to the enzyme (Huber & BeMiller, 2000).

XI.3.2 Metabolism by Colonic Flora

The abilities of the MGP Ingredients PDSP products Fibersym® 70 (a wheat-starch based product, since renamed Fibersym® RW) and Fibersym® 80 ST (potato-starch based) to be fermented by the colonic microflora were evaluated *in vitro* by Dr Lillian Thompson of Toronto University (Annex XI-A). Raw starches were cooked and enzymatically predigested under conditions simulating the small intestine and the residue recovered. Essentially 74% of wheat-based PDSP and 64% of potato based PDSP remained undigested. The recovered starches were then used as substrates for *in vitro* fermentation by faecal preparations derived from a healthy human. Gas production and short chain fatty acid production were both measured over a 24 hour period. Both starches were fermented to a similar degree as measured by either gas production or short chain fatty acid production (Table XI.1). In terms of short chain fatty acid production the predominant acids produced were (in descending order) acetic, butyric and propionic acids. Small amounts of formic, isobutyric, isovaleric and valeric acid were also produced, but these were subsequently metabolised and lost. Consequently they were not detected after 24 hours fermentation. Allowing for the inevitable variation in individual faecal microflora seen; when comparing data from these types of experiments (McBurney & Thompson, 1989); the results obtained by Thompson for either wheat starch or potato starch derived PDSP are consistent with those obtained for naturally occurring starches as determined by McBurney *et al* (1990). However it should be noted that for both resistant starches the molar proportion of butyrate formed was higher than seen with amylose, or wheat starch.

Apart from PDSP, other modified starches have also been assessed for their ability to be metabolised by the colonic microflora. As in the case of unmodified RS_2 and RS_3 resistant starches; the fermentability of the resistant starch can change with chemical modification. Wang *et al* (2002) investigated the effects of modifying the chemical structure of amylose on the faecal microflora of female Balb/C, SPF mice fed diets containing specified starches. They also studied the resistant starches' potential prebiotic effects - following co-administration of the probiotic *Bifidobacterium* sp. Lafti B8. Essentially they compared the effects of acetylated and carboxymethylated amylose with that of the parent starch. The trial also included mice fed a diet containing non resistant (waxy) starch and a control cohort (mice fed a commercial synthetic diet). No difference in weight gain was seen in any of the groups, while in comparison with the commercial diet, diets containing waxy starch or amylose promoted increased growth of *Bifidobacterium* spp., *Lactobacillus* spp. and Coliforms. With regard to the chemically modified starches, different effects were seen; carboxymethylated amylose brought about only a slight increase in the numbers of *Bifidobacterium* spp., while in the case of the acetylated starch the increase was comparable

to the other starches. In contrast, while carboxymethylated starch had a similar effect on Coliforms present within the faecal flora, feeding of acetylated starch reduced the numbers of these organisms.

Table XI-1 ***In vitro*** **microbial fermentation of the MGP Ingredients Phosphated Distarch Phosphate Products Fibersym® 70 (now renamed Fibersym® RW) & Fibersym® 80 ST using human faecal inocula (Annex XI-A) together with a comparison of short chain fatty acid data with a previously published study (McBurney *et al*., 1990)**

	Gas Production		
Substrate	**Initial rate (mmol per hour)**	**Total production after 24hours (mmol)**	**Short chain fatty acid production (after 24 hours): molar ratio C_1:C2:C_3:C_4**
Fibersym® 70 (wheat)	19.5	214.2	0.00:0.59:0.19:0.23
Fibersym® 80 ST (potato)	19.5	214.2	0.00:0.58:0.20:0.22
In comparison with data from McBurney et al. (1990)			
Wheat starch			0.00:0.65:0.16:0.19
Kidney bean starch			0.13:0.58:0.16:0.13
Rice starch			0.02:0.57:0.17:0.23
Maize			0.00:0.58:0.19:0.23
Amylopectin			0.03:0.60:0.22:0.15
Amylose			0.31:0.44:0.13:0.13

Prebiotic studies showed that inclusion of either amylose or the carboxymethylated amylose had a significant prebiotic effect on the establishment of *Bifidobacterium* Lafti 8B over and above that seen with the control or the waxy starch diets (acetylated starch not reported). In common with the high amylose diet, the faecal SCFAs contents were shifted towards the production of butyrate. The molar ratio of the acids (acetate:propionate:butyrate) for mice fed a standard diet (control) was 0.69:0.19:0.12. Moving the mice to a high starch diet led to increased faecal starch excretion and increased amounts of short chain fatty acids in the faeces. More starch and short chain fatty acids were found in the faeces of mice fed the resistant starch diets - the greatest increase being seen in mice fed the amylose containing diet. The faecal SCFA molar ratio's found were: for the waxy starch diet (0.72:0.21:0.07); this contrasted with 0.60:0.15:0.25 for mice fed the amylose diet and 0.55:0.18:0.27 for the diet containing carboxymethylated starch.

Similar work by Annison *et al* (2003), studied SCFA production in rats fed maize starch or its acylated derivatives (acetylated, propylated or butyrylated) for 14 days. Acylation increased the amount of starch present in the caecal contents by over 10-fold and the total SCFA present there by over 3-fold. Acylation also affected the molar ratios of the SCFA produced. Thus for acetylated maize starch the ratio (acetate:propionate:butyrate) was 0.11:0.20:0.69; this compared with 0.46:0.42:0.12 and 0.47:0.15:0.38 for propylated and butyrylated starches respectively. Further work by the same group (Bajka *et al.*, 2006), using maize-derived amylose or its butyrylated derivative demonstrated that not only did butyrylation of starch increase SCFA (and butyrate) production in the rat caecum but also made the starch more resistant to the increased susceptibility to amylolysis associated with cooking of the starch.

XI.3.3 Human Studies

Effects on General Health & Bowel Function

Pieters *et al* (1971) described a study where 12 healthy volunteers each consumed 60g of PDSP on each of 4 successive days. No adverse effects were reported. Faecal outputs were normal in terms of consistency, quantity and frequency. Faecal analysis revealed no differences in terms of lactic acid or faecal water contents.

Subsequently a double blinded cross-over feeding study using crackers was performed at the University of Nebraska (Martínez *et al.*, manuscript submitted, Annex XI-B). In each of three 3-week periods 10 young adults consumed a low resistant starch diet or one containing 30 – 33 g per day of either an RS_2 or RS_4 as the PDSP Fibersym® RW. Generally both high RS diets were well tolerated by the subjects. No significant changes in faecal pH were observed and, with the exception of a possible mild increase in flatulence associated with either RS, no adverse effects were observed (Table XI-2), In terms of effects on the large bowel microflora, in four of the ten subjects consumption of crackers containing PDSP led to approximately an order of magnitude increase in the numbers *Bifidobacteria* spp. present in the faeces. This compared with two individuals eating the RS2 diet (Table IX-3) exhibiting a similar type of increase. Small increases in the numbers of *Bacteroides* spp. were also seen.

More recently, studies have been undertaken to evaluate the effects of chemically modified starches on glucose and insulin metabolism. Raben *et al.* (1997) described a study where healthy male subjects (n = 11) were fed meals consisting of a vanilla pudding (i.e. starch was cooked and gelatinised) made with different types of potato starch (native, acetylated or with added cyclodextrin) within a fruit sauce. Modification of the starch failed to have any effect on dietary thermogenesis or on the oxidation of carbohydrate, fat or protein. Consumption of the pudding based on the acetylated starch led to altered kinetics with regard to post prandial -glycaemia and –insulinaemia, with reductions in area under the curve (AUC) values for both parameters. The authors concluded that modification of the starch in this manner led to improved satiety, glycaemic and insulinaemic responses.

Table XI-2 Symptomatic responses of subjects fed low and high RS diets. (Annex XI-B)[1].

Symptom	RS2	Fibersym® RW	Control	None
Bowel Movement	1.72 ± 0.83	1.90 ± 0.93	1.73 ± 0.77	1.74 ± 0.59
Stool Consistency	2.07 ± 1.29	2.23 ± 0.92	2.03 ± 0.91	2.00 ± 0.83
Discomfort	1.65 ± 0.65	1.87 ± 0.79	1.50 ± 0.55	1.54 ± 0.43
Flatulence[2]	2.42 ± 1.28	2.27 ± 1.00	1.37 ± 0.58	1.54 ± 0.43
Abdominal pain	1.63 ± 0.79	1.47 ± 0.67	1.40 ± 0.60	1.36 ± 0.62
Bloating	1.67 ± 0.98	1.40 ± 0.52	1.07 ± 0.14	1.29 ± 0.45

1 Responses were graded on a scale of 1 (best) to 5 (worse)
2 Significant differences were detected by ANOVA ($P < 0.05$). Tukey's post hoc test did not detect significance in pair wise comparisons

Glycemic Index Laboratories Inc. (Annex XI-C) studied the effects of Fibersym® 70 (now known as Fibersym® RW), on the glycaemic and insulinaemic responses of healthy individuals. Subjects (both sexes, age range 18 –75 years: BMI < 30) fasted overnight and then ate each of three meals on separate occasions. Each meal consisted of muffins made with or without the modified starch plus one of a selection of beverages of the subject's choice. Muffins were made with the same amount of either conventional wheat starch or the modified version. Two of the meals eaten were controls (142g or 68g of muffin, made with conventional starch) and the third meal was of a muffin made from the modified starch (143g final weight). For the control muffins, no statistically significant meal-related differences in plasma glucose area-under-the curve (AUC) or peak rise data were seen. In the case of the insulinaemic responses, plasma insulin AUC values with a blunter response was seen when subjects ate the smaller (68g) muffin. The results for the muffin made with Fibersym® 70 (143g) were intermediate between the two control meals.

In terms of plasma insulin peak rise, increasing the amount of control meal eaten led to a significant increase in this parameter. Values obtained for the test muffin were again intermediate between the two. It was concluded that some of the modified starch was actually digested and absorbed (similar blood glucose data), however this occurred at a slower rate than seen with the equivalent control meal (blunter insulin response). The lack of differences in blood glucose data between the two control meals was noted and it was suggested that this may be due to the high fat and protein content of the muffins. Studies such as those by Reader *et al*. (2002) and Hertzler & Kim (2003) have both shown that the protein and fat content of foods with different carbohydrate contents can influence both glycaemic and insulinaemic responses. Another contributory explanation may be that as Nugent (2005) has proposed and as discussed in section XI-2.4.1 in general resistant starches have a more powerful effect in attenuating post-prandial insulinaemia than glycaemia.

Table XI-2 Quantification of bifidobacteria through genus specific qRT-PCR (quantitative real time polymerase chain reaction) in faeces of subjects fed low and high RS diets. (Annex XI-B)

Subject	Log 10 bifidobacteria cells/g faeces (mean ± SD)			
	RS2	Fibersym® RW	Control	None
1	10.56 ± 0.40§	11.00 ± 0.12**§§§	10.12 ± 0.13	9.95 ± 0.22
2	9.48 ± 0.31	9.53 ± 0.24	9.40 ± 0.50	9.39 ± 0.17
3	7.29 ± 0.48	6.39 ± 0.51	6.49 ± 0.37	7.10 ± 0.92
4	10.62 ± 0.29	10.98 ± 0.28*§§	10.26 ± 0.44	10.21 ± 0.22
5	10.47 ± 0.24	10.44 ± 0.84	10.53 ± 0.24	10.62 ± 0.26
6	11.22 ± 0.24**§§	11.42 ± 0.14***§§	10.34 ± 0.22	10.59 ± 0.27
7	10.46 ± 0.35	10.83 ± 0.55	10.06 ± 0.42	10.29 ± 0.44
8	9.91 ± 0.57	10.48 ± 0.10	9.51 ± 0.44	9.94 ± 0.85
9	10.60 ± 0.31	11.19 ± 0.24§	10.52 ± 0.38	10.33 ± 0.31
10	10.52 ± 0.10	10.23 ± 0.15	10.09 ± 0.10	9.77 ± 0.51
ALL	10.11 ± 1.09*	10.25 ± 1.46**§	9.73 ± 1.20	9.82 ± 1.03

* Significantly different to control (P<0.05)
** Significantly different to control (P<0.01)
*** Significantly different to control (P<0.01)
§ Significantly different to none (P<0.05)
§§ Significantly different to none (P<0.01)
§§§ Significantly different to none (P<0.01)

Fibersym® RW was subsequently evaluated in another food (a cereal bar) at Kansas State University where the puffed wheat component was replaced by the resistant starch (Al-Tammini *et al*, manuscript in preparation, annex XI-D). Nine (5 females, 4 males) healthy, older adult volunteers (age, 68 ± 6 years; BMI 25 ± 4 kg m^{-2}) consumed on separate occasions isoglucidic amounts of a glucose drink, or one of two cereal bars made with either puffed wheat or PDSP. Each meal was consumed on two separate occasions. Post-prandial glycaemia following consumption of either cereal bar, both in terms of peak glucose response and area under the curve was lower than seen with the glucose meal. Both cereal bars were considered to have 'low' GI values (< 55, Brand-Miller *et al.*, 2003*)*, however that of the bar made with PDSP (GI value: 36 ± 16) was lower than that of the cereal bar made with puffed wheat (53 ±24).

The cereal-bar data re-emphasises the point that the effects of PDSP, like other resistant starches on the entero-insular axis are mediated in part by other factors including the formulation and processing of the food it is delivered in.

XI.4 SUMMARY

A certain proportion of starch eaten by humans, remains undigested in the small intestine and passes into the colon. This starch has been described as resistant starch. Within the colon it can be fermented by the resident colonic microflora or voided with the faeces.

Based on a number of factors, in particular, physical location within plant tissue, physico-chemical properties, molecular organisation or whether or not they have been chemically modified, resistant starches fall into one of four groups ($RS_{1\rightarrow4}$). Irrespective of its form, resistant starch appears to exert its most potent physiological effects by virtue of it being a substrate for the colonic microflora, leading to the production of short chain fatty acids. Using the most widely current and accepted definitions, resistant starch can be considered to be a form of dietary fibre.

PDSP, being a chemically modified starch, falls into the RS_4 group. Data presented here demonstrates that PDSP and in particular Fibersym® RW behave no differently from either naturally occurring resistant starch (RS_1 and RS_2) nor resistant starch formed as a process of physical processing (e.g. cooking – RS3). Evidence for this statement includes the observations that:

1. In common with other resistant starches their ability to withstand amylolysis decreases once gelatinisation of the starch granules has taken place.
2. It is well tolerated when fed in doses within acute studies.
3. Under *in vitro* conditions Fibersym® RW & Fibersym® 80 ST are both metabolised by the faecal microflora in a similar manner to other starches in terms of the molar ratios of short chain fatty acids, which are produced. Phosphorylation of two starches of different botanical origins thus did not adversely affect a key physiological property of resistant starches, *viz.* the ability to be fermented by colonic bacteria to produce SCFA.
4. When fed to humans PDSP can modify the colonic microflora in a similar manner to RS2 preparations.
5. When incorporated into muffins that would be eaten by the consumer, PDSP had a greater effect on postprandial insulinaemia than it did on parallel measurements of glycaemia. However when incorporated into a different food (cereal bars) PDSP was observed to significantly reduce post-prandial glycaemia. This has also been seen in the cases for other resistant starch products.

XII MICROBIOLOGICAL INFORMATION ON THE NOVEL FOOD

Application of Decision Tree

	Question	Answer	Comments
1.	"Is the presence of any micro-organisms or their metabolites due to the novelty of the product/process"	No	As demonstrated in the preceding text the manufacture of PDSP does not involve the use of any microbially-based process. Any presence of micro-organisms is consequential to their being in the source raw material (wheat).

XII MICROBIOLOGICAL INFORMATION ON FIBERSYM® RW and FIBERRITE® RW PHOSPHATED DISTARCH PHOSPHATE

Consideration of Section II demonstrates that no novel microbially-related process is involved in the manufacture of either Fibersym® RW or FiberRite® RW.

Reference to Section I addresses questions of the microbiological specifications for the products and the ability of the manufacturer to comply with them.

XIII TOXICOLOGICAL INFORMATION ON THE NOVEL FOOD

Application of Decision Tree

	Question	Answer	Comments
2.	"Is there a traditional counterpart to the novel food that can be used as a baseline to facilitate the toxicological assessment?"	No	See answers to subsequent questions
3.	"Is there information from a range of toxicological studies appropriate to the novel food to show that the novel food is safe under anticipated conditions of preparation and use?"	Yes	Toxicological data for phosphated distarch phosphate exists and is summarised in section XIII.2 Additional supporting toxicological evidence as to the safety of phosphated distarch phosphate with regard to contribution made by the presence of phosphorus is detailed in XIII.3; while the physiological relevance of resistant starch is discussed in XIII.4.
4.	"Is there information which suggests that novel food might pose an allergenic risk to humans?"	Yes	Points 4 and 5 are discussed in XIII.5.
5.	Is there sufficient information to allow the potential allergenicity of the novel food to be monitored	Yes	
6.	Has the level of allergenicity been determined in controlled trials	No	Point 6 is addressed under current EU labelling regulations (Commission Directive 2007/68/EC) and discussed in XIII.5

XIII TOXICOLOGICAL INFORMATION ON PHOSPHATED DISTARCH PHOSPHATE (PDSP)

XIII.1 INTRODUCTION

PDSP is an approved additive (E1413) and has undergone extensive safety evaluation studies. Its safety in use can be assessed by toxicological trials previously undertaken (reviewed in section XIII.2) using product derived principally from maize starch. Additional factors that have to be taken into account are the potential physiological consequences of increased phosphorus (section XIII.3) and resistant starch intakes (previously discussed in Section XI) associated with consumption of PDSP. Inclusion of additional information relating to the safety of phosphorus and other resistant starches (chemically modified or otherwise) assists in addressing deficiencies in the knowledge concerning the safety of PDSP itself.

XIII.2 PHOSPHATED DISTARCH PHOSPHATE (PDSP)

XIII.2.1 Background

Reviews undertaken by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) on the safety and appropriateness of PDSP for food use were undertaken over a number of meetings (JECFA, 1962, JECFA, 1972, JECFA, 1970$_a$ JECFA, 1974$_a$, JECFA, 1979). The expert committee's final view (JECFA 1982a) was that it should set an acceptable daily intake of 'not specified' for the material This term was defined as follows:

"The statement "ADI not specified" means that, on the basis of the available data (toxicological, biochemical, and other), the total daily intake of the substance, arising from its use or uses at the levels necessary to achieve the desired effect and from its acceptable background in food, does not, in the opinion of the Committee, represent a hazard to health. For this reason, and for the reasons stated in individual evaluations, the establishment of an acceptable daily intake (ADI) in mg/kg bwt is not deemed necessary."

The committee went on to state:

"The ADI includes distarch phosphate prepared using trimetaphosphate or phosphated distarch phosphate or the sum of both. Subject to limits of phosphorus load (see monograph on "Phosphoric acid and phosphate salts")."

XIII.2.2 Biochemical Studies

Starch-Hydrolysis

As discussed in Section X1.3.2; PDSP is more resistant to amylolysis than native starch (Kohn & Kay, 1963$_a$; Leegwater, 1971) *in vitro.* Studies by Sieb's group (Woo and Seib, 2002; Shin *et al.*, 2004) demonstrated that *in vitro* susceptibility to amylolysis depended on the physical state of the starch. Pre-gelatinising starch made it more susceptible to enzymic breakdown. Irrespective of whether pregelatinised or not, phosphorylation increased resistance of starch to amylolysis in a dose dependent manner. Such an increase could be achieved with starches of different botanical origin.

In vitro Fermentation

The fermentability of PDSP using starch sourced from two different botanical sources Fibersym® 70 (since renamed Fibersym® RW and wheat-starch based) and Fibersym® 80 ST (potato-starch based) by faecal bacteria has been demonstrated (Section, XI.3, Thompson; Annex XI-A). As discussed in Section XI.3, the SCFA results obtained compared well with previously published data for wheat starch (McBurney *et al*., 1990).

XIII.2.3 Absorption Distribution Metabolism and Excretion (ADME)

In vivo digestibility experiments have been undertaken by Kohn and Kay (1963b) and de Groot and Spanjers (1970). Kohn and Kay fed groups of 10 male rats daily with 5g basal diet plus 1, 2 or 4g native (maize) or modified starch for a period of 10 days. Over that period, neither abnormal behaviour nor differences in weight gain were observed. In a subsequent study, de Groot and Spanjers (1970) fed groups of 10 male and 10 female rats low residue diets containing 0, 25 or 50% modified or native starch for 7 days followed by a low residue diet with an added 4% cellulose for a further 3 days. No abnormal behaviour during the study was observed (this included the absence of diarrhoea). Faecal output was normal however there was an increase in faecal dry matter in the test versus the control groups. At the end of the study; small, dose dependent, reductions in weight gain associated with the amount of modified starch present were observed. On autopsy caecal enlargement was seen in animals fed diets containing the modified starches; however this was not accompanied by changes in histopathology.

XIII.2.4 Acute Feeding Trials

No acute toxicity data is available for PDSP however data does exist for a related compound distarch phosphate. In its monograph on starch diphosphate JECFA (1974_b) reported that LD_{50} values following oral administration had been determined by Hodge (1954, 1956). These are detailed in Table XIII-1.

In its assessment of Hodge's data, JECFA observed that despite the small numbers of animals used, no deaths were reported and that histopathological examination of the livers and kidneys removed from guinea pigs, rabbits and cats indicated an absence of abnormalities associated with treatment.

Table XIII-1 Acute Toxicity (LD_{50} Values) of Distarch Phosphate following Oral Administration (after JECFA, 1974)

Species	LD_{50} (g per kg body weight)	Reference
Mouse, female	> 24 000 > 19 000	Hodge (1954) Hodge (1956)
Rat, female	> 20 000 >35 000	Hodge (1954) Hodge (1956)
Guinea Pig	> 8 800 >18 000	Hodge (1954) Hodge (1956)
Rabbit	> 7 000 >10 000	Hodge (1954) Hodge (1956)
Cat	> 6 800 >9 000	Hodge (1954) Hodge (1956)

XIII.2.5 Subchronic Feeding Trials

Subacute feeding trials of PDSP have been performed in three species (rat, dogs and pigs).

Rats

Kohn et al. (1964_a) described a 60-day feeding trial where groups of 10 male and 10 female rats were fed a control diet or diets containing a progressively increasing amount of PDSP (range: 10 - 30% PDSP). Four test and two control animals died during the trial due to factors unrelated to the diet. Apart from this, all animals behaved normally. Female rats fed the test diets showed reduced weight gain compared with the controls. No differences were seen in haematological or urine analysis data between test and control groups. Although the liver weights of males and kidney weights of both sexes were lower than the controls, no abnormalities were found on gross or histopathological examination.

Subsequently, De Groot and Spanjers (1970) performed a 56-day study using diets containing 0, 25 or 50% PDSP. This was performed with groups of 10 male and 10 females fed each diet. No detectable effects on behaviour or weight gain were seen, nor were any incidences of diarrhoea observed. Although there were indications of increased faecal water content in both male and female rats fed the 50% PDSP diet, the variation was so great that it was not considered statistically significant. At autopsy a slight increase in filled caecal weight was seen in male rats fed the 25% PDSP diet, this was not considered to be of toxicological significance.

Kohn *et al*., 1964_b also undertook a 90-day feeding trial using groups of 25 male and 25 female rats diets containing 0.2, 1.0 and 5% PDSP or unmodified (maize) starch. Due to an outbreak of intercurrent disease within the rat

colony, 11 controls and three test animals died during the trial. No abnormal behaviour associated with the test substance was observed. Weight gains for all groups were comparable. No differences in organ weights or gross or histopathological appearance of tissues were observed. A similar finding was obtained for haematological and urine analyses.

Dogs — Cervenka and Kay (1963) administered PDSP encapsulated in gelatine to groups of 3 male and 3 female beagle dogs for 90 days. The dose rates were 50, 250 or 1250 mg PDSP per kilogram bodyweight per day. No adverse effects with regard to behaviour, mortality, weight gain, organ weights, gross or histopathology of the tissues, haematology, blood chemistry, urine analysis, liver function or organ weights were seen.

Pigs — Anderson et al. ($1973_{a,b}$) fed weanling Pitman-Moore miniature pigs formula diets containing either 5.4% native (maize) starch or 5.6% PDSP for 25 days. No abnormal behaviour was observed during the trial and food intakes and weight gains were similar. At the end of the study, no differences in blood (haemoglobin) and serum biochemistry (cholesterol, triglyceride, calcium, phosphorus, alkaline phosphatase, urea nitrogen, total protein, albumin and globulin) were seen. Autopsy examination revealed no differences in relative organ weights, nor the carcass (water, fat, protein, ash, calcium, phosphate sodium and magnesium) and liver (water, fat, protein and ash) composition.

XIII.2.6 Chronic Feeding Trials

Chronic feeding trials with PDSP have been performed in rats (de Knecht-van Eekelen *et al.*, 1971; de Groot *et al.*, 1974) over a 104 week period. Groups of 30 male and 30 female rats were fed diets containing 0, 5, 10 or 30% PDSP. Over the two-year period, no adverse effects associated with diet in terms of behaviour, general appearance, mortality, food intake growth rate or food efficiency were seen. No consistent diet related effects were observed with regard to haematology, serum chemistry and urine analyses. At autopsy, no evidence of carcinogenic effects was seen and few dietary effects were observed. Males fed the highest dose of PDSP exhibited lower relative spleen weights compared with controls, while in the case of females fed the same diet both spleen and kidney weights were significantly higher. No differences in caecal weights due to diet were observed. Examination of the kidneys from all groups revealed the occurrence in some animals of focal hyperplasia of the renal papillary and pelvic epithelium, accompanied by calcified patches in the underlying tissue. These lesions often protruded into the renal pelvis and were localised most often in the papilla near the junction of the papillary and pelvic epithelium. Although observed in all dietary groups the lesion was more pronounced in rats fed the highest dose of PDSP.

The toxicological significance or otherwise of this type of kidney lesion was reviewed by Roe (1974) and discussed further in a paper by Hodgkinson et al (1982) who looked at other chemically modified starches (acetylated distarch phosphate and acetylated distarch adipate). These and other data were also considered by the Scientific Committee for Food (Commission of European Communities, 1982). In summary the committee's observations were that, renal calcification manifested itself in three major forms: pelvic nephrocalcinosis, corticomedullary calcinosis and calculus formation. All of these lesions appeared to be a consequence of imbalances in dietary levels of calcium, magnesium, phosphorus and/or

vitamin B_6. Together with another kidney condition (acute tubular nephropathy), all four conditions are frequently observed in untreated laboratory rats, particularly in older rats. The incidence of these conditions was also considered to be sex and strain dependent.

In its assessment, the Committee observed that consumption of many carbohydrates which fail to be digested in the jejunum (including sugars such as lactose, sugar alcohols and complex carbohydrates such as resistant starches) are associated with caecal enlargement and bacterial breakdown of these carbohydrates in the caecum and ileum. Some of the metabolites produced by this process are subsequently absorbed into the body. This process is associated with increased calcium absorption, which, in turn, can result in increased calcium excretion. The Committee went on to observe that the rat seemed exquisitely sensitive to these effects and the phenomena were less often seen in mice fed chemically modified starches and was absent in hamsters. It went on to conclude that pelvic nephrocalcinosis was something peculiar to the rat as the most sensitive species and was of little relevance to the safety assessment of modified starches in man.

XIII.2.7 Reproductive Toxicology

Multigeneration trials have also been performed with PDSP (Til *et al* 1971; de Groot *et al.*, 1974). Groups of male (n = 10) and female (n = 20) rats were fed diets containing starch modified with either sodium trimetaphosphate (0.01%) residual phosphorus) or sodium polyphosphate (0.35% residual phosphorus). They were mated at 12 *(= litter a)* and 20 weeks (= *litter b)*. Progeny from litter b (F_{1b} and F_{2b}) were used as parents for the succeeding generation. The F_{3b} generation were fed the same diet for a further 3 weeks after weaning and then subjected to autopsy. No adverse effects in terms of appearance, behaviour, body weights, fertility, litter size, resorption quotients, weights of pups and mortality were seen. Although the filled caecal weights of F_{1b} parent males and the weights of spleens from F_{3b} females were found to be higher than controls; gross and microscopic examination of the tissues revealed no abnormalities.

XIII.2.8 Mutagenicity and Genotoxity Studies

No information is currently available with regard to short-term mutagenicity or genotoxicity studies of PDSP or other modified starches.

XIII.2.9 Human Studies

Human studies relating to PDSP have been discussed in Section XI.4. Pieters *et al* (1971) described a study where 12 healthy volunteers each consumed 60g of PDSP on each of 4 successive days. No adverse effects were reported. Faecal outputs were normal in terms of consistency, quantity and frequency. Faecal analysis revealed no differences in terms of lactic acid or faecal water contents. Subsequently Martinez *et al* (Annex XI-B) carried out a double blind feeding trial where 10 subjects were fed a control (low RS) diet or diets providing over 30g per day of either a commercial RS2 or Fibersym® RW. These diets were well tolerated with only a slight increase in the degree of flatulence reported for both RS diets.

In terms of human metabolism, studies by Raben *et al*. (1997) with acetylated modified starch, showed that, when eaten in a food, this starch did not have any effect on dietary thermogenesis nor on the oxidation of carbohydrate, fat or protein. However it did result in a bluntening of both post-prandial -glycaemic and –insulinaemic responses.

Glycemic Index Laboratories Inc. (Annex XI-C) studied the effects of Fibersym® 70 (since renamed as Fibersym® RW, manufactured by MGP Ingredients Inc. and the subject of this application) in muffins on the glycaemic and insulinaemic responses of healthy individuals. Individuals ate meals either of control muffins (142 or 68g portions) or the same product but made with the modified starch (portion size 143g) No statistically significant meal-related differences in plasma glucose area-under-the curve (AUC) or peak rise data were seen. In the case of the insulinaemic responses, plasma insulin AUC values for muffins made with the modified starch were significantly lower than for the equivalent dose of control muffin but still higher than seen for the 68g control muffin meal. In terms of plasma insulin peak rise, reducing the amount of control meal eaten led to a significant reduction. These results were consistent with both the formulation of the muffin and other studies using resistant starches (discussed in section XI.4). Food matrix plays an important role in the effects of PDSP on postprandial glycaemia and insulinaemia. Further work undertaken by Al-Tamini and Haub at Kansas State University (Annex XI-D) demonstrated that inclusion of Fibersym® RW in a cereal bar led to significant reductions in both parameters.

XIII.2.10 Summary

A number of studies have been undertaken to evaluate the safety of PDSP, most of them in the rat, although short-term human trials have also been performed. Details of the type/duration of study performed, the species of animal evaluated (where applicable) and reference are given in Table XIII.2. PDSP has also been shown to behave like naturally occurring resistant starches in particular RS_2 by virtue of the facts that it fermented by faecal micro-organisms (and by inference the colonic microflora) and that it elicits similar effects on post-prandial glycaemia and insulinaemia, depending on the type of food it is incorporated into.

Table X111-2 Toxicological Evaluation of PDSP – Summary of Data Available

Type of Study	Species	Study Details	Reference
In vitro digestibility	Not Applicable	Sensitivity to pancreatic alpha amylase	Kohn & Kay (1963_a)
		Sensitivity to porcine pancreatin and intestinal mucosal preparation	Leegwater (1971)
		Effect of phosphorylation and or gelatinisation on presence of RDS, SDS & RS	Woo & Seib (2002) Shin et al (2004)
In vitro microbial digestibility	Human faecal microflora	Gas and short chain fatty acid production measured over 24 hours	Thompson (Annex _)
In vivo digestibility	Rats (male)	5g basal diet plus 1g, 2g, or 4g native or PDSP for 10 days	Kohn & Kay (1963_b)
	Rats	Low residue diets containing 0.25 or 50% modified starch for 5 days followed by low residue diet with 4% cellulose for a further 3 days	De Groot *et al.* (1970)
Subacute Feeding Trials	Rats	Diets containing 10 progressively increasing to 30% native starch or PDSP over 60 days.	Kohn *et al.* ($1964_{a)}$
		Diets containing 0, 25 or 50% native starch or PDSP for 56 days	de Groot *et al* (1970)
		Diets containing 0.2, 1.0 or 5.0% native starch or PDSP for 90 days	Kohn *et al* (1964_b)
	Pitman-Moore miniature Pigs	Formula diets containing 5.4% native starch or 5.6% PDSP for 25 days	Anderson *et al.* ($1973_{a,b}$)

Table X111-2 Toxicological Evaluation of PDSP – Summary of Data Available

Type of Study	Species	Study Details	Reference
	Beagle Dogs	PDSP in gelatine capsules, administered at 50, 250 or 1250 mg per kg body weight per day for 90 days	Cervenka & Kay (1963)
Chronic Feeding Trials	Rat	Diets containing 0, 5, 10, 30% PDSP for 104 weeks	de Knecht-van Eekelen *et al* (1971); de Groot *et al* (1974)
Reproductive Toxicology	Rat	Diets containing 10% PDSP during and after gestation over 3 generations	Til *et al* (1971) de Groot *et al* (1974)
Tolerability	Human volunteers	60g PDSP per day for 4 successive days	Pieters *et al* (1971)
Glycaemic and insulinaemic responses	Human volunteers	Studies with acetylated starch	Raben *et al.* (1997)
		Muffin-based meals containing Fibersym® RW	Glycemic Index Laboratories Inc. (Annex _)
		Cereal bars containing Fibersym® RW	Al Tamimi *et al.* (Annex IX-C)

XIII.3 PHOSPHATE/PHOSPHORUS

XIII.3.1 Introduction

Consumption of PDSP might contribute to an increase in dietary phosphorus consumed either due to free phosphate residues carried over in the manufacturing process or as a consequence of PDSP hydrolysis leading to the liberation of phosphate into the digesta.

Phosphorus as phosphate is an essential nutrient for life. In determining its reference nutrient intake (RNI) value, the Committee on Medical Aspects of Food Policy (Department of Health, 1991) determined it should be equal to that for calcium on a molar basis. Thus the same document quoted an RNI value of 17.5mmol (700mg) calcium per day for healthy (non-lactating) adults. The corresponding phosphorus RNI was set at 550mg per day. For calcium intakes that differed from the RNI, the committee recommended that phosphorus intake should be the molar equivalent of the amount. The Scientific Panel on Dietetic Products Nutrition and Allergies (European Food Safety Authority, 2005) recently reviewed the

question of phosphorus, and observed that the average intake from foods within the European Union lay between 1 000 and 2 000mg per day.

The safety of phosphates in food has been considered by JECFA has been reviewed on a number of occasions (JECFA, 1965, 1967, 1970$_a$, 1971, 1974$_c$,1982$_b$)

XIII.3.2 Absorption, Digestion, Metabolism and Excretion (Human)

The human metabolism of phosphate was most recently reviewed by the European Food Safety Authority in 2005. Phosphorus is absorbed from the digesta in the form of the inorganic phosphate ion and takes place mainly in the jejunum either by a saturable, active-transfer mechanism, which uses 1,25-dihydroxy vitamin D as well as by passive diffusion (Chen *et al*. 1974). Absorption efficiencies are estimated to be between 55-70% in humans (Nordin, 1976) and between 65 and 90% in children (Ziegler and Formon, 1983). As in the case of calcium, extracellular concentrations of phosphate are subject to endocrine regulation via the parathyroid hormone (PTH) and 1,25-dihydroxy vitamin D. Some phosphate is not as tightly regulated as serum calcium and follows a circadian cycle (Portale *et al*,., 1989; Calvo *et al.*, 1991).

The production of PTH is also stimulated by imbalances in the levels of calcium and phosphorus, for example under conditions of low calcium and high phosphorus consumption. This leads to a reduction in serum phosphorus and a consequent increase in urinary phosphate excretion (Calvo *et al.*, 1988, 1991). Phosphorus excretion occurs mainly through the kidneys with 80-90% being reabsorbed (European Food Safety Authority 2005). Recent work has shown that renal excretion of phosphorus is regulated by a novel group of phosphaturic hormones (phosphatonins) rather than simply by PTH and 1,25 dihydroxy vitamin D.

XIII.3.3 Acute Feeding Trials

Data (LD_{50} and LD_{100}) for acute feeding trials is summarised in Table XIII-3 below. These refer to inorganic sources of phosphorus which already exist as or can form the phosphate ion *in vivo*. The list includes phosphoric acid, phosphates, ortho-phosphates, tetra sodium diphosphates, triphosphates, polyphosphates and calcium phosphate. The results from these acute studies indicate that phosphorus in the form of inorganic phosphates has a low toxicity.

XIII.3.4 Subchronic Feeding Trials

Subchronic feeding trials have been undertaken for a wide number of phosphates in diverse species. These are summarised in Table XIII-4. At high doses of phosphate, adverse effects were seen. These manifested themselves primarily as growth retardation and kidney damage including nephrocalcinosis.

Table XIII-3 Acute Toxicity (LD_{50} Values) of Phosphorus Containing Salts following Oral Administration (after JECFA, 1982_b)

Compound	Species	Oral LD_{50} (mg/kg body weight)	Estimated Oral LD_{50} (mg phosphorus equivalents/ kg body weight)	Reference
Phosphoric acid salts				
Sodium dihydrogen phosphate (NaH_2PO_4)	Guinea pig	2 000	517	Eichler (1950)
	Mouse	3 700	956	Food & Drug Research Laboratories Inc. (1975_a)
	Rat	4 100	1 059	
Potassium dihydrogen phosphate (KH_2PO_4)	Mouse	3 200	729	Food & Drug Research Laboratories Inc. (1975_b)
	Rat	2 820	642	
Monocalcium phosphate ($CaHPO_4$)	Mouse	4 600	1049	Food & Drug Research Laboratories Inc. (1975_c)
	Rat	2 170	495	
Pyrophosphates				
Disodium dihydrogen pyrophosphate ($Na_2H_2P_2O_7$)	Mouse	3 350	936	Food & Drug Research Laboratories Inc. (1975_a)
	Rat	1 690	472	
	Hamster	1 660	464	
Tetrasodium pyrophosphate ($Na_4P_2O_7$)	Mouse	1 300		Food & Drug Research Laboratories Inc. (1975_c)
	Rat	1 380		
Tri- & Polyphosphates				
Sodium triphosphate ($Na_5P_3O_{10}$)	Mouse	2 380	603	Food and Drug Research Laboratories Inc. 1973_b
	Rat	1 700	430	
	Rabbit	2 500	634	

Table XIII-3 Acute Toxicity (LD_{50} Values) of Phosphorus Containing Salts following Oral Administration (after JECFA, 1982_b)

Compound	Species	Oral LD_{50} (mg/kg body weight)	Estimated Oral LD_{50} (mg phosphorus equivalents/ kg body weight)	Reference
Sodium hexa-Metaphosphate ($Na_6P_6O_{18}$)	Mouse Rat	3 700 2 400	1 125 729	Food and Drug Research Laboratories Inc. 1975_d

Table XIII-4 Subchronic Feeding Trials: Summary of Results Obtained (after JECFA, 1982_b)

Compound	Species	Study Details	Outcome	Reference
Phosphoric acid salts				
Potassium dihydrogen phosphate	Rats	3 groups of 12 rats fed a diet with/without KH_2PO_4 giving different ratios of calcium to phosphorus %Ca: %P Control 0.56 0.42 'normal' 0.47 0.43 'high' 0.50 1.30 over 150 days	Experimental observations made at 50, 60 & 150 days. No adverse effects on behaviour nor growth seen. No adverse clinical signs, nor changes in gross- or histo- pathology	Dymsa *et al.* (1959)
Dicalcium phosphate or ammonium polyphosphate	Sheep	Groups of 5 sheep fed diets with/without either additional 11g dicalcium phosphate or 6.225g ammonium polyphosphate over 7 days	Supplementing with phosphate led to increased appetite, weight gain and apparent nitrogen retention	Fishwick (1974)
Pyrophosphates				
Tetrasodium pyrophosphate or sodium monophosphate	Rats	Groups of 34-36 young rats fed diets containing 0, 1.8%, 3.0% & 5.0% pyro-phosphate or mono-phosphate for 6 months	Normal growth seen in rats the 1.8-% pyro-phosphate and mono-phosphate diets. Growth in the other treatment groups	Hahn & Seifen (1959); Hahn *et al*. (1958)

Table XIII-4 Subchronic Feeding Trials: Summary of Results Obtained (after JECFA, 1982$_b$)

Compound	Species	Study Details	Outcome	Reference
			retarded. Main toxic effect in rats fed the 3.0 & 5% phosphate diets was nephrocalcinosis	
		Groups of 20 male & 20 female rats fed diets containing 0, 2.5%, & 5.0% pyrophosphate or 5.0% monophosphate for 16 weeks	Normal growth seen up to 2.5% diet. Increased kidney weights seen in females and impairment of kidney function (males) observed at diets containing 2.5 & 5%.	Datta *et al.* (1962)
			Kidney damage (calcification and necrosis) seen at 2.5% with increasing severity as dosage was increased.	
Tri- & Polyphosphates				
Sodium hexameta-phosphate or sodium tripoly-phosphate	Rat	Groups of 5 rats fed diets containing 0.2, 2 and 10% of either salt for 1 month. Controls fed standard diet or standard diet with 10% sodium chloride or 5% sodium mono-phosphate,	Inhibition of growth with diets containing 10% phosphate or chloride – no deaths. Increased kidney weights and tubular necrosis seen. In 2% groups, although growth was normal, kidney damage was also noted - however the inflammatory changes were different from those seen in the 10% groups.	Hodge (1956)
	Dog	1 dog fed dose of 0.1g/kg/day. 2 dogs fed daily doses rising from 1.0g/kg/day at the beginning to 4.0g/kg/ day at end (5 months)	Weight-loss seen in dog fed increasing doses of sodium hexametaphosphate at dose of 2.5g/kg/day, dog fed tripoly-phosphate only exhibited weight loss at 4.0g/kg.day. Urinalysis, haematology and organ weights were all normal. Dog fed	Hodge (1956)

Table XIII-4 Subchronic Feeding Trials: Summary of Results Obtained (after JECFA, 1982$_b$)

Compound	Species	Study Details	Outcome	Reference
			increasing doses of sodium tripoly-phosphate developed hypertrophy of left ventricle. Kidney tubular damage seen in dogs fed the highest doses however none seen in those fed at the 0.1% level	
Commercial Kurrol's salt preparation (1/3 Kurrol's salt & 2/3 mixture disodium and tetrasodium phosphates	Rat	Groups of 10 male and 10 female rats fed diets containing 0.5, 1, 2.5 & 5% of the mixture fed for 12 weeks	Retardation of growth only seen in rats fed the 5% diet. Normal kidney weights seen in the 0.5% group, these rose progressively with the dose. Nephro-calcinosis and calcification of other tissues seen in rats fed the 5% diet. Less damage was seen in the 2.5% group and none in the 0.5% group.	van Esch *et al.* (1957): van Genderen *et al* (1958)
Ammonium polyphosphate	Pig	Substitution of phosphorus in de-fluorinated rock phosphate with 50 or 100% ammonium polyphosphate	No differences in feed intake and feed efficiency seen compared with controls	Clawson & Armstrong (1981)

XIII.3.5 Chronic Feeding Trials

The number of chronic feeding trials performed on phosphates has been far more limited. These are summarised in Table XIII-5. Adverse effects (growth retardation and kidney damage) were only seen in animals fed the highest doses.

XIII.3.6 Reproductive Toxicology

A number of reproductive toxicology trials have been performed using various phosphate salts in different animal models. In no cases were maternal toxicity or teratogenic effects seen. A summary of these trials is provided in Table XIII-6

XIII.3.7 Mutagenicity and Genotoxity Studies

A number of mutagenicity trials have been performed with various phosphorus salts none of the compounds were to be mutagenic in any of the systems used. A summary of the salts evaluated and the test systems used is provided in Table XIII-7

Table XIII-5 Subchronic Feeding Trials: Summary of Results Obtained (after JECFA, 1982_b)

Compound	Species	Study Details	Outcome	Reference
Phosphoric acid & its salts				
Phosphoric acid	Rats	3 successive generations fed diets containing 0, 0.4 or 0.75% phosphoric acid	Apart form increased rates of dental attrition in test groups no adverse effects (including no changes in acidosis or calcium metabolism) seen	Lang (1959)
Tri- & Polyphosphates				
Mixture of 1/3 Kurrol's salt & 2/3 disodium di-hydrogen pyrophosphate	Rats	Groups of 10 males & 10 females fed Sherman diets containing 0, 0.5, 1.0, 2.5 & 5.0% mixture over 3 generations	Adverse effects on growth and fertility only seen with rats fed diets containing 5% mixture. Dose dependent nephrocalcinosis seen in animals fed diets containing 1.0% mixture and above.	van Esch *et al.* (1957)
Sodium tripolyphosphate	Rats	2 year feeding trial, groups of 50 male and 50 female rats fed diets containing 0, 0.05, 0.5 & 5% sodium tripoly-phosphate.	Adverse effects on growth and survival plus low-grade anaemia only seen in 5% groups. This was accompanied by increased kidney weights and renal calcification. No adverse effects seen in other groups. Reproduction studies with the 0 and 0.5% Groups over 3 generations showed no impact on performance.	Hodge (1960_a)

Table XIII-5 Subchronic Feeding Trials: Summary of Results Obtained (after JECFA, 1982$_b$)

Compound	Species	Study Details	Outcome	Reference
Sodium hexameta-phosphate	Rats	2 year feeding trial, groups of 50 male and 50 female rats fed diets containing 0, 0.05, 0.5 & 5% sodium hexametaphosphate	High mortality seen in all groups (including controls). Adverse effects (growth retardation & kidney damage) only seen in 5.0% treatment group Reproduction studies with the 0 and 0.5% groups over 3 generations showed no impact on performance.	Hodge (1960$_b$)

Table XIII-6 Reproductive Toxicology & Teratology Studies: Summary of Results Obtained (after JECFA, 1982$_b$)

Compound	Species	Study Details	Outcome	Reference
Phosphoric acid salts				
Sodium dihydrogen phosphate	Rats	24 pregnant albino (Wistar) rats treated by oral intubation days 6-15 of gestation (dose up to 410mg/kg body weight/day; equivalent to 106 *mg phosphorus*/ kg body weight/day)	No evidence of maternal or foetal toxicity nor teratogenesis seen at any dose.	Food & Drug Research Laboratories Inc. (1975$_a$)
Potassium di-hydrogen phosphate	Mice	24 pregnant CD-1 mice treated by oral intubation on days 6-16 of gestation (dose up to 370mg/kg body weight/ day; equivalent to 96 *mg phosphorus*/ kg body weight/day)	No evidence of maternal or foetal toxicity nor teratogenesis seen at any dose.	Food & Drug Research Laboratories Inc. (1975$_b$)
Calcium hydrogen phosphate	Rats Mice	Methods of treatment as Food & Drug Research Laboratories Inc. (1975$_a$). Top doses (rats & mice respectively) 282 & 320 mg/kg body weight/day (equivalent to 64 & 73 *mg phosphorus*/ kg body	No evidence of maternal or foetal toxicity nor teratogenesis seen at any dose.	Food & Drug Research Laboratories Inc. (1973$_c$)

Table XIII-6 Reproductive Toxicology & Teratology Studies: Summary of Results Obtained (after JECFA, 1982$_b$)

Compound	Species	Study Details	Outcome	Reference
		weight/day respectively).		
	Rats Mice	Methods of treatment as Food & Drug Research Laboratories Inc. (1975$_a$). Top doses: (rats & mice respectively) 410 & 465 mg/kg body weight/day (equivalent to 93 & 106 *mg phosphorus*/ kg body weight/day respectively).		
Pyrophosphates				
Sodium acid pyrophosphate	Rats Mice Hamsters Rabbits	Methods of treatment for rats & mice as Food & Drug Research Laboratories Inc. (1975$_a$). In the case of hamsters, between 22 & 25 female golden hamsters were orally dosed from day 6 through day 10 of gestation. For rabbits, between 20 & 22 female Dutch Belted Rabbits were orally dosed between days 8 and 18 of gestation. Top doses: 169; 335, 166 & 128 mg/kg body weight/day (rats, mice, hamsters & rabbits). This was equivalent to 89, 176, 87 & 67*mg phosphorus*/ kg body weight/day respectively).	No evidence of maternal or foetal toxicity nor teratogenesis seen at any dose.	Food & Drug Research Laboratories Inc. (1975$_c$)
Tri- & Polyphosphates				
Sodium hexa-metaphosphate	Rats Mice	Methods of treatment as Food & Drug Research Laboratories Inc. (1975$_a$). Top doses: (rats & mice respectively) 240 & 370 mg/kg body weight/day (equivalent to 72 & 111 *mg phosphorus*/ kg body weight/day respectively).	No evidence of maternal or foetal toxicity nor teratogenesis seen at any dose.	Food & Drug Research Laboratories Inc. (1975$_d$)

Table XIII-6 Reproductive Toxicology & Teratology Studies: Summary of Results Obtained (after JECFA, 1982$_b$)

Compound	Species	Study Details	Outcome	Reference
Sodium tripoly-phosphate	Rats Mice Hamsters Rabbits	Methods of treatment as Food & Drug Research Laboratories Inc. (1975$_a$, 1975$_c$). Top doses: 170, 238, 141, 250 mg/kg body weight/day (rats, mice, hamsters & rabbits). This was equivalent to 43, 60, 63, 36 *mg phosphorus* /kg body weight/day respectively).	No evidence of maternal or foetal toxicity nor teratogenesis seen at any dose.	Food & Drug Research Laboratories Inc. (1975$_d$)

Table XIII-7 Phosphates: Absence of Demonstrable Mutagenicity and Genotoxicty – Details: Summary of Systems Studied (after JECFA, 1982$_b$)

Compound	Test System	Mammalian Metabolic Activation	Reference
Phosphoric acid salts			
Monocalcium phosphate Monopotassium phosphate Monosodium phosphate	Mutagenicity *Saccharomyces cerevisiae* strain D4 *Salmonella typhimurium* strains TA – 1535, TA – 1538, TA – 1538	 With & without With & without	Litton Bionetics Inc (1975$_{a,b,c}$)
Pyrophosphates			
Sodium acid pyrophosphate	Mutagenicity *S. typhimurium* TA-1530 *S. typhimurium* TA-1535, TA-1536, TA-1537, TA-1538 Genotoxicity *S. cerevisiae* strain D3	 Host mediated assay (mice) With and without Host mediated assay (mice)	Newell *et al.,* 1974

Table XIII-7 Phosphates: Absence of Demonstrable Mutagenicity and Genotoxicty – Details: Summary of Systems Studied (after JECFA, 1982[b])

Compound	Test System	Mammalian Metabolic Activation	Reference
	Dominant lethal tests (rats)		
	Translocation test (rats)		
Tetrasodium pyrophosphate	Mutagenicity		
	S. cerevisiae strain D4	With & without	Food & Drug Research Laboratories 1975[d]
	S. typhimurium strains TA–1535, TA–1538, TA–1538	With & without	
Polyphosphates			
Sodium tripolyphosphate	Mutagenicity		
	S. typhimurium strains TA–1530 & G-46,	Host mediated assay (mice)	Litton Bionetics Inc (1974)
		(In vitro) with or without	
	Genotoxicity		
	S. cerevisiae D3	Host mediated assay (mice)	
		(In vitro) With or without	
	Rat bone marrow cells (*in vivo)*		
	Human lung cells *(in vitro)*		
	Dominant lethal test		
Sodium hexametaphosphate	Mutagenicity		
	Salmonella typhimurium strains TA – 1535, TA – 1538, TA – 1538	With or without	Litton Bionetics Inc (1975[d])

XIII.3.8 Human Studies

Early studies by Leichsenring *et al.* (1951) investigated the effects of consuming additional calcium and phosphorus in 6 women. Subjects were fed a basal diet containing 300mg calcium and 800mg phosphorus (molar ratio, Ca:P, 1:3.4), and then transferred to diets containing 1500mg calcium and 800mg phosphorus (molar ratio, Ca:P 1:0.7) or 1500mg calcium and 1400mg phosphorus (molar ratio, Ca:P, 1:1.2). Increasing the amount of phosphorus in the second test diet led to a reduced utilisation of calcium compared with the first. A similar study was undertaken by Malm (1953) in 4 males fed a basal diet of 400mg calcium and 1400mg phosphorus (molar ratio Ca:P, 1:4.5) and then fed them an additional 750mg phosphorus (as phosphoric acid) for 1 week. This resulted in a slight decrease in urinary calcium excretion. Increasing the treatment period to 12 weeks led to a further reduction in urinary calcium excretion. One caveat that has to applied to these studies was that the calcium:phosphorus molar ratios of the basal diets exceeded the 1:1 ratio currently recommended (Department of Health, 1991). Subsequent studies in subjects eating more normal diets indicated that phosphate was well tolerated. Lauersen (1953) observed that consumption in of 2000 – 4000mg phosphoric acid by male students for between 10 and 14 days revealed no observable change in urinary excretion patterns suggestive of disturbed mineral metabolism. Similar results were published in the same paper for sodium dihydrogen phosphate ($NaH_2PO_4.2H_2O$) which was tolerated with no adverse effects, when consumed at 6000mg per day for 15 days. Lang (1959) reported similar results for sodium dihydrogen phosphate.

Zemel and Linkswiler (1981) investigated the effects of phosphate (potassium dihydrogen phosphate or sodium hexametaphosphate) supplementation against a background of low and high calcium diets. The basal (low calcium) diet contained 399mg calcium and 835mg phosphorus (molar ratio 1:2.), this was supplemented with either phosphate to provide a phosphorus content of 1835mg (molar ratio Ca:P, 1:59). The high calcium diet contained 1194mg calcium and 1835mg phosphorus (molar ratio Ca:P, 1:2). Eight male subjects were fed each of the diets within the context of a 4x4 Latin square design. The diets were well tolerated. Increasing the amount of dihydrogenphosphate in the diet diminished the negative calcium balance effects associated with a low calcium diet. Sodium hexametaphosphate had no effect.

Calvo *et al* (1988) performed dietary studies to investigate the effects of a high phosphorus, low calcium diet, typical of that eaten by many teenagers and young adults in the US. Groups of eight men and eight women (ages 18-25 years) were fed a diet based on commercially available foods and providing approximately 820mg calcium and 930mg phosphorus (molar ratio Ca:P, 1:1.5). After eight days, the subjects were switched to a similar type of diet, but one containing 420mg calcium and 1660mg phosphorus (molar ratio Ca:P, 1:4.9). Moving to the low calcium, high phosphorus diet led to increases in parathyroid hormone, serum phosphorus and plasma 1,25-dihydroxyvitamin D and both urinary hydroxyproline and cAMP excretion in both sexes. In women decreased serum ionised- and total- calcium levels were also observed.

Similar studies were reported by Whybro *et al.* (1998). They examined the effects of phosphate supplements on calcium homeostasis and bone turnover in young men (19 –38 years). Two studies were described. In the first, subjects (n = 10) took an additional 1000mg phosphorus against a background diet containing 800g of both calcium and phosphorus for one week. This changed the molar Ca:P ratio from 1:1.3 to 1:2.9. In the second study (n = 12)

the additional phosphorus dose was incrementally increased from 0 through 1000, 1500 and 2000mg; equivalent to molar Ca:P ratios of 1:1.3; 1:2.9; 1:3.7 and 1:4.5. In this case subjects were held on each treatment regime for one week. In study 1, additional phosphorus consumption led to increased urinary phosphorus excretion and decreased urinary calcium excretion. Serum parathyroid hormone levels also rose. However no changes in serum phosphate and osteocalcin or urinary N-telopeptide excretion were seen. In study 2, at the top phosphate dose used (molar Ca:P ratio, 1:4.5), while there was an increase in urinary phosphate and a decrease in urinary calcium excretion no changes in urinary phosphorus, parathyroid hormone or urinary deoxyproline excretion were observed. From these results, the authors concluded that in young men, phosphate supplementation did not affect bone turnover.

The situation in young women is less clear. Grimm *et al* (2001), studied a cohort of 10 healthy women. The subjects consumed a control diet containing 1400mg calcium and 1700mg phosphorus (molar ratio Ca:P, 1:1.6) for 4 weeks. Subjects were then transferred to a diet containing 1995mg calcium and 3008mg phosphorus (molar ratio Ca:P 1:1.9). Supplementing with phosphorus at this level was observed often to cause intestinal discomfort (abdominal distress, soft stools and/or mild diarrhoea). The intervention was observed to lead to increases in serum parathyroid hormone levels and decreased serum osteocalcin. Urinary excretion of creatine in urinary deoxypyridinoline and creatinine in pyridinoline were both increased; while urinary microalbumin excretion was reduced. None of these changes were considered to be biologically significant. No changes were seen in circulating levels of calcium, phosphorus (as phosphate), zinc and 1,25 dihydroxyvitamin D. Urinary beta-2-microglobulin excretion was also unaffected. The authors concluded that under the study conditions used, no significant changes in bone-related hormones and pyridinum cross links – markers of bone resorption and renal function in young women were found.

These results contrast with those of Lamberg-Allardt's group. Early work by this group (Karkainnen & Lamberg-Allardt, 1996) investigated the effect of acute doses of phosphate (single dose of 1500mg phosphorus or three successive doses, each of 500mg phosphorus) in female volunteers. Calcium and bone metabolism were then monitored for a 24 hour period. In the case of calcium metabolism markers, serum phosphorus increased under both regimes, while serum calcium levels fell only when the dose was administered in three parts. Urinary excretion of calcium decreased and serum parathyroid hormone rose in both treatment groups. With regard to bone formation, of the three markers studied, no changes in serum osteocalcin were seen. On the other hand, serum levels of bone specific alkaline phosphatase activity decreased with either treatment while administration of the single dose led to reduction in serum levels of carboxy-terminal propeptide of type I collagen. In terms of bone resorption markers (serum carboxy-terminal telopeptide of type I collagen and urinary deoxypyridinoline excretion) no treatment related effects were seen. The authors concluded that under the conditions used, high doses of phosphate might inhibit the early stages of bone formation in young women.

Subsequent work (Kemi, *et al.*, 2006) in young females (n = 14), investigated the effects of increasing consumption of phosphorus supplements (0, 250, 750 or 1500mg per day) taken over 4 day periods against a reference diet background, which provided 250mg calcium and 495mg phosphorus (molar ratio Ca:P, 1:1.5). Serum, calcium levels were seen to decrease only at the highest dose of phosphorus used. However dose dependent increases in serum parathyroid hormone and the bone resorption marker, N-terminal telopeptide of collagen type

I were seen. These were accompanied by a dose dependent reduction in the bone formation marker bone-specific alkaline phosphatase. Recent work from this group (Karp, *et al*. 2007) has shown the importance of food-matrix effects. Young healthy women aged between 20 and 30 years were fed a base diet containing about 250mg calcium and 500mg phosphorus (molar ratio Ca:P 1:1.5). This was consumed either on its own or supplemented with an additional 1000mg phosphorus in one four forms, 1000mg from either meat, cheese, whole grains or an inorganic phosphate supplement. Increases in serum parathyroid hormone levels were only seen when the subjects took the additional phosphorus in the form of inorganic phosphate. Consumption of meat was observed to increase markers of both bone formation and resorption. Eating cheese led to reduced serum parathyroid hormone levels and markers of bone resorption.

Interventions in older women have also been reported Studies by Martini and Wood (2002) looking at the effectiveness of different dietary interventions on calcium nutrition in the elderly found that a five fold difference (101 *vs.* 536mg) in the amount of phosphorus present in the calcium supplement had no effect on a range of clinical parameters induced by those supplements associated with calcium metabolism.

More recently attention has focussed on the roles played by dietary phosphate on the endocrine system, particularly with respect to factors associated with impaired renal function and osteoporosis. As discussed in XIII.3.2, it has long been shown that imbalances in calcium and phosphorus intakes lead to changes in the circulating level of the parathyroid hormone PTH (Goldsmith, 1976). Early work by Goldsmith *et al* (1968) demonstrated that multiple myeloma `patients (n = 14) receiving phosphate supplements of between 1000 and 2000mg phosphorus as part of their therapy for periods of up to 15 months reported reduced bone pain and a reduction in urinary calcium excretion was also seen. This was not accompanied by extra-skeletal calcification. One patient exhibited pedal and pretibial oedema, which receded once phosphate treatment was discontinued. Another, exhibited dyspepsia, but this was also observed when the subject consumed the placebo or other medication. Goldsmith's group went on to investigate the effects of phosphorus supplementation, using buffered phosphate preparations (equivalent to approximately 1g phosphorus per day) in postmenopausal women suffering from osteoporosis (Goldsmith *et al.,* 1976). Administration of the supplement did not elicit any changes in PTH nor serum calcium levels. Four of the subjects were studied using balance techniques. In these cases administration of the supplement led to the calcium balance shifting towards the positive. The density of the distal radius changed variably, however that of the midradius increased slightly in all patients.

At about the same time, as part of investigations into the potential effects of journeys into outer space of long duration; Hulley *et al* (1971) investigated the role of dietary phosphate in the onset and development of disuse osteoporosis. Five healthy young men (aged 19 – 27) were subjected to bed rest for 24-30 weeks. During the first 12 weeks both urinary and faecal excretion of calcium increased, this was accompanied by losses of central calcareous material. Consumption of phosphate supplements (1327mg phosphorus (as a mixture of K_2HPO_4 and KH_2PO_4) led to a reduction in hypercalciurea and improvement in calcium balance (however losses of central calcareous material continued).

Further work in Mexican postmenopausal women (Mendez *et al*., 2002) determined that dietary phosphorus intakes were not a determinant in the risk of developing osteoporosis. Other workers have shown that phosphorus can have a beneficial effect on bone structure in osteoporotic individuals either singly in combination with other treatments. Marie and Caulin

(1986) evaluated the effects of three different interventions (calcitonin (50IU x 5 days every three weeks); phosphate (1.5g per day) or; combined therapy) against a group of double blind placebo controls for 6 months. Beneficial effects to bone formation (increase in both bone-forming surfaces and bone matrix production) in the iliac crest were only seen in subjects taking the oral phosphate preparation. A combination of calcitonin and phosphate gave the best result (22.1% increase in the thickness of the trabeculae and 31.1% increase in trabecular bone volume). Similar results were obtained by Cantatore *et al.* (1987). In one study they administered to nineteen patients with radiological and clinical evidence of osteoporosis oral phosphorus (1000mg for 10 days, followed by calcitonin (100 MRC U per day) for 20 days. A further group of 6 women were administered oral calcium (1000mg per day). Treatment with phosphate and calcitonin led to a significant increase in serum osteocalcin and parathyroid hormone. No variation in the controls was observed. A second, similar, trial revealed that this treatment also led to increased levels of circulating 1,25 dihydroxyvitamin D. The authors considered that these data suggested that phosphorus treatment was a useful activator of bone formation and its improvement of serum 1,25 dihydroxyvitamin D levels of benefit to the treatment of osteoporosis.

With regard to the role of 1,25 dihydroxyvitamin D a note of caution should be made. Subsequent work by Brot *et al*, (1999) working with a cohort (n = 510) of perimenopausal women observed that within normal physiological ranges raised levels of serum 1,25 dihydroxyvitamin D, were associated with decreased bone mineral density and content, a reduced dietary calcium:phosphorus ratio and increased bone turnover. Portale *et al.* (1984) observed that in children with moderate renal insufficiency, restriction or supplementation with dietary phosphorus led to changes in circulating and 1,25-$(OH)_2$ vitamin D and PTH levels. Essentially restricting phosphorus intake (from 1200mg per day to 350mg phosphorus per day) led to a reduction in PTH and an increase in 1,25-$(OH)_2$ vitamin D. Increasing (from 1200mg to 2400mg phosphorus) the amount of phosphorus present had the opposite effect.

XIII.5 POTENTIAL ALLERGY CONCERNS & CONSUMER ADVICE

Wheat is considered to make a significant contribution to adverse reactions towards food within the population. The two principal manifestations of dietary significance being the enteropathy coeliac disease (including the related condition of dermatitis herpetiformes), which involves an immune responses to gluten); as well as true (IgE-mediated) wheat allergy (Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment, 2000). Current European legislation (Commission Directive 2007/68/EC) specifies a list (Annex III-a) of foods with allergenic potential, which when present in a food, must have a clear reference to the source allergen made on the label. This requirement extends to conventional wheat starch and would *de facto* apply in the case of Fibersym® RW and FiberRite® RW (the subjects of this application). There is therefore a legal obligation on any food manufacturer to declare the presence of these ingredients clearly on the label. Inclusion of modified wheat starch in the form of PDSP would not therefore be expected to contribute any greater risk to wheat intolerant consumers than commercially available wheat starch already used within the food industry.

In accordance with the Food Standards Agency letter to the European Commission (Food Standards Agency, 2009), pending experimental evidence to demonstrate to the contrary manufacturers of foods containing either Fibersym® RWor FiberRite® RW will be

advised that they should precautionary label these products to the effect that they may have laxative effects in young children.

CHAPTER 5: DISCUSSION

Preamble

The purpose of the chapter is to argue the case that modified wheat starch is physiologically a resistant starch and that it is safe for its intended uses.

JUSTIFICATION THAT MODIFIED WHEAT STARCH CAN BE REGARDED AS A RESISTANT STARCH

It is now recognised that a proportion of starch consumed by humans remains undigested and passes into the colon. There, it can act as a substrate for the indigenous microflora. This material is referred to as resistant starch. Resistant starch can be further classified into four categories (Englyst & Cummings, 1987; Baghurst *et al.*, 1996). These are:

RS_1 - Starch resistant by virtue of its physical inaccessibility (e.g. coarsely milled grains);
RS_2 - Starch present in a granular form, which is particularly resistant to digestion (e.g. as found in potatoes, bananas and amylose-rich starch granules);
RS_3 This is retrograded material formed during the cooling of gelatinised (cooked) starch and which has a acquired a glass-like structure;
RS_4 – Chemically modified starches that may or may not be fermented in the colon.

Applying the most commonly used definitions of dietary fibre (American Association of Cereal Chemists, 2001; Agence Française de Sécurité Sanité des Aliments, 2002;; Health Council of the Netherlands, 2006; Codex Alimentarius Commission, 2007; European Food Safety Authority, 2007), resistant starches can be categorised as forms of dietary fibre in that:

- They are carbohydrate polymers with a degree of polymerisation greater than 3;
- They are resistant (partly or totally) to amylolytic attack and therefore escape digestion within the small intestine
- Consequently they pass into the large intestine, where they are partially or totally metabolised by the colonic microflora
- The secondary metabolites produced, in particular SCFA, confer additional benefits to the host including trophic effects on the colonic epithelium, and reductions in the pH of the colonic lumenal contents.

It must also be recognised that the degree to which starch is resistant to digestion within the small intestine is dependent on how the food is processed. As discussed previously (Answers to Question XI), food-processing leads to starch gelatinisation and a consequential increase in susceptibility to enzymic degradation. This applies to a number of resistant starches and has been shown to be of significance *in vivo* (e.g. Wunsche, *et al.*, 1987; Englyst and Cummings, 1987).

The physiological benefits ascribed to resistant starch relate primarily to its prebiotic effects (Topping & Clifton, 2001; Topping *et al.*, 2003). In terms of effects on post prandial glycaemia and insulinaemia, its effects seem to be moderate and more directed at modifying post-prandial insulin levels, both in terms of peak amounts and also area under the curve

(Nugent, 2005). The insulin blunting effect is dependent on the degree of processing, the more processed the resistant starch the less the blunting effect (Brown *et al,* 2003).

For PDSP to be considered as a resistant starch it is therefore necessary to demonstrate that it is:

- To a significant degree resistant to amylolysis *in vitro;*
- Capable of being fermented by the colonic microflora, leading to the formation of SCFA;
- Effective in modulating the entero-insular axis, when consumed in a food as opposed to the purified raw form.

Modified wheat starches and in particular Fibersym RW® and FiberRite® RW meet these criteria for the reasons discussed below.

Susceptibility or Otherwise to Attack by α-Amylase

Early work (Kohn & Kay, $1963_{a;}$ Leegwater, 1971) demonstrated that PDSP was more resistant to amylolysis by either porcine pancreatic α-amylase (or a mixture of porcine pancreatin and small intestinal mucosal preparations. Recent work published by the laboratory of Seib has shown that the degree of resistance to α-amylase attack is dependent on the level of phosphorylation and that gelatinisation of the starch diminished this resistance, however this effect became less pronounced with increased levels of phosphorylation (Woo and Seib, 2002).

Fermentability by Faecal (Colonic) Microflora

A number of modified starches have been assessed for their ability to be fermented by the colonic microflora, in animal systems (Wang *et al,* 2002; Annison *et al.* 2003). Given that the microflora of rodents is different to that of humans, it is preferable to demonstrate that PDSP can be metabolised by the indigenous microflora of the human colon. As discussed in section XI.3.2, work undertaken by Dr. Lillian Thompson (Toronto University) has demonstrated that PDSP sourced from either wheat or potato starches is fermented by the faecal microflora of a healthy human being and that significant quantities of SCFA are produced (Annex XI-A). Evidence from Martínez *et al.* (Annex XI-B) indicates that PDSP has a similar probiotic effect to RS_2 products.

Effects on The Entero-Insular Axis

Applying the conclusions of Nugent (2005) and observations such as those of Brown *et al* (2003) with amylose and Raben *et al.* (1997) with other types of modified starch; PDSP would be expected to at least modulate post-prandial insulinaemia when given as a cooked food. Studies with muffins containing % weight Fibersym® 70 by Glycemic Laboratories Inc. (Annex XI-C) have demonstrated that this indeed is the case. Further work (Al-Tamimi *et al*, Annex XI-D) using a different food (cereal bars) have shown that PDSP can also reduce post prandial glycaemia).

SAFETY IN USE

Safety as a Modified Starch

Studies with both PDSP and distarch phosphate in various animal species under subchronic, chronic and developmental/reproductive study conditions have shown no adverse responses (discussed in answer to question XIII). One potential issue was the question of pelvic nephrocalcinosis sometimes seen in rats fed these materials. This question was addressed by the Scientific Committee for Food in 1976 and 1981 (Commission of the European Communities, 1976; 1982). In reviewing the literature and other studies at its 13th meeting; the committee were of the opinion that this condition was relatively common in laboratory rats, particularly older ones. Strain- and sex- related differences were also observed. Furthermore, many sugars and other carbohydrates (including modified starches) which escaped digestion in the small intestine were fermented in the caecum. Fermentation promoted caecal enlargement and the absorption of bacterial metabolites into the blood stream. This uptake was also accompanied by an increased absorption of calcium and consequential increased urinary calcium excretion. The committee considered that pelvic nephrocalcinosis was a rat-related phenomenon and not of relevance to human safety.

As discussed above, physiologically, PDSP behaves in a similar manner to a naturally occurring RS_2-type starch. Using UK dietary record data (Section IX) and assuming that all foods applied for were supplemented with Fibersym® RW at the maximum applied for the highest amount consumed was for male teenagers (average 15.2 g/day, with intakes of 20, 32.8 and 48.7 g/day at the 90, 95 and 97.5 percentile points – Table IX-3). These estimated extreme intakes are lower than the 60g/day used in a tolerance study in humans where no effects were observed (Pieters *et al.*, 1971) and comparable with the levels used by Martínez *et al.* (Annex XI-B).

PDSP is currently permitted for use *quantum satis* with the exception of products intended to be weaning foods for infants and children of good health, where a limit of 50g kg^{-1} has been set (European Parliament and European Council, 1995). It should be noted that this application does not include this particular group of foods.

Safety in Terms of Contribution to Dietary Phosphorus Intakes

The safety of phosphorus (as phosphates) in foods has been the subject of debate. Various bodies have considered what should be considered as a upper safe level of intake. These include the Joint WHO/FAO Expert Committee on Food Additives (JECFA, 1974$_c$), the Institute of Medicine (1997), the Expert Group on Vitamins and Minerals (2003) and the European Food Safety Authority (2005).

Using a rat-based model, JECFA, estimated that a safe upper limit for phosphorus intake was in the order of 6 600mg day.$^{-1}$ This was based on the lowest level at which nephrocalcinosis had been observed and acknowledged that rats were not only uniquely sensitive to this condition but also that the response was highly variable (discussed above). They proposed an acceptable daily intake (ADI) of total dietary phosphorus (from both natural and added sources) to be in the range 0 to 70 mg per kg body weight. These analyses were reviewed by the Institute of Medicine (1997) in the US. Based primarily on animal studies (typified by

diets with higher phosphorus densities than found in humans) they identified a number of pathological conditions (e.g. changes in calcium-regulating hormones, altered calcium uptake and changes in skeletal structure) of concern. Nevertheless the Institute stated that it could not find evidence of adverse effects due to excess phosphorus in healthy individuals. However raised levels of blood phosphate and consequential adverse effects had been seen in people suffering from certain clinical conditions, for example end-stage renal disease and vitamin D intoxication. For healthy adults and adolescents (overall age range 9 through 70); the Institute calculated an upper limit of 4.0g phosphorus per person per day and for children (1 – 8 years) and the elderly (>70 years). The cohort calculated to have the highest phosphorus intakes were males aged 14-18 years (2.5g phosphorus per day), this was still well below the upper limit.

The Expert Group on Vitamins and Minerals (2003) reviewed data concerning the safety of phosphorus – primarily from the point of view of food supplements. They considered that there were insufficient data to establish a safe upper limit. The committee also noted the work of Brixen *et al*. (1992), which had shown that high intakes of phosphorus could bring about detrimental changes in the parathyroid hormone control of calcium homeostasis. Additionally, that individuals suffering from vitamin D deficiency and consuming high levels of phosphorus were at additional risk of hyperparathyroidism. Applying a safety factor of three (for inter-individual variability) to the No Apparent Observed Effect Level of 750mg per day obtained by Brixen *et al.* (1992), the expert group recommended a guidance level of 250 mg supplemental phosphorus per day. These findings were further considered by the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (2004). The committee considered both the group's report and additional new information, not made available for the 2003 report. The committee concluded that any influence on the calcium-parathyroid hormone axis was of a transient nature, representing short-term adaptive changes and did not necessarily suggest an adverse effect of phosphorus on bone health. However the committee did not discount the possibility that under conditions of low calcium/vitamin D intakes, excess phosphorus could exacerbate bone calcium resorption effects.

The Committee's views were endorsed by the European Food Safety Authority (European Food Safety Authority, 2005) in their opinion regarding setting a tolerable upper limit for the consumption of phosphorus. The view expressed by the European Food Safety Authority was that there was insufficient evidence to set a tolerable upper limit. In its risk characterisation, European Food Safety Authority considered that normal healthy individuals could tolerate intakes of up to, at least, 3 000mg per day without exhibiting adverse effects. However there were reports that some individuals exhibited mild gastrointestinal symptoms (e.g. osmotic diarrhoea, nausea and vomiting) if exposed to supplemental doses of >750mg per day. In common with the Committee on Toxicity, European Food Safety Authority did not consider that there was sufficient data to conclude that high phosphorus intakes could aggravate hyper-parathyroidism in calcium and/or vitamin D compromised individuals. Estimated current intakes of phosphorus in European countries suggested an average phosphorus intake from both food and supplements to be in the region of 1 000 to 1 500mg per day (97.5 percentile: ~ 2 600mg). Consideration of Table IX-6 shows that under the most extreme case, estimated additional phosphorus consumption form either PDSP would be greatest in male teenagers (average 80mg with intakes of: 105, 173 and 256 mg/day at the 90, 95 and 97.5 percentile points) however these values would be considerably lower than those currently being considered by the Authority.

IMPACT ON CONSUMER NUTRITION AND SAFETY

Nutrient Content & Nutritional Declarations

Staple foods containing naturally resistant starch as an additional source of dietary fibre are already on the market. For example private label white bread sold by the supermarket J.S Sainsbury includes Himaize® which results in the products having dietary fibre contents over twice that seen with products made only with white flour (www.himaize.co.uk; www.sainsbury.co.uk). Based on the technical data publicly available this would be equal to somewhere in the region of 10% added resistant starch product per 100g finished product.

In terms of nutritional labelling the Food Standards Agency recommends that dietary fibre information should be based on the AOAC gravimetric methods of analysis (Food Standards Agency, 2004). A number of other points should also be remembered:

- Best practice requires that any nutritional information supplied on a food product label is derived from analysis rather than calculation (although this is also permitted), this applies particularly to dietary fibre.
- The carbohydrate value declared is 'available carbohydrate' i.e. refers to carbohydrates capable of being digested within the human small intestine and excludes any carbohydrate determined to be dietary fibre as measured by AOAC validated methodology.

Compliance with current Food Standards Agency recommendations in the labelling of foods made with modified wheat starch as an ingredient will ensure that the consumer can make informed decisions concerning how much dietary fibre and available carbohydrate they are consuming. Use of modified wheat starch in foods would not therefore be expected to compromise those for whom the available carbohydrate of the foods that they eat was critical to their health (e.g. type I diabetics).

Impact on Other At-Risk Groups

As discussed in Chapter 4 under Section XIII.5, European Community legislation requires consumers to be advised of the presence of wheat starch, if used as an ingredient within a food product. The same requirement applies to both Fibersym® RW and FiberRite® RW, no increased risk to wheat intolerant individuals is therefore anticipated.

With regards to the risk of side effects due to abnormal bowel function, as shown in Table XI-1; the molar ratios of short chain fatty acids produced by Fibersym® RW is comparable with that published for unmodified wheat. Recent studies have shown that the human colonic microflora rapidly adapts to resistant starch such that faecal samples taken from weanlings were found to be capable of fermenting resistant starch (Scheiwiller *et al,* 2006). Resistant starch has been proposed for use in cholera therapy both in adults and adolescents (Ramarkrisha *et al.,* 2000), as well as infants and young children (Rahhupathy *et al.,*2006).

CONCLUDING OBSERVATIONS

PDSP has a long history of safe use within the European Community. There is a large body of evidence to demonstrate that PDSP generally and both Fibersym® RW and FiberRite® RW are resistant starches. The fact that they are derived from wheat would not present any additional risk to either sufferers from gluten-related enteropathies (e.g. coeliac disease) or wheat allergy. Current legislation requires food-manufacturers to label appropriately when wheat, or wheat derived products (including wheat starch) are used. Replacement of unmodified starches or other chemically modified (i.e. not phosphorylated) starches with either of these products would not therefore, place an increased burden to the public health due to food-allergy concerns. The same holds for any potential increase in supplemental phosphorus intakes.

On the basis of the available toxicological information, its equivalence to other (chemically unmodified) resistant starches and level of supplemental phosphate intake; it is proposed that use of either Fibersym® RW or FiberRite® RW would present no additional risk for human health on the bases of their proposed additional uses as novel foods.

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ANNEXES TO THE SUBMISSION