APPLICATION FOR THE APPROVAL OF RS4-FIBRE MODIFIED STARCH (PHOSPHATED DI-STARCH PHOSPHATE) FROM HIGH AMYLOSE MAIZE STARCH

FINAL

NON-CONFIDENTIAL

Regulation (EC) No 258/97 of the European Parliament and of the Council of 27th January 1997 concerning novel foods and novel food ingredients

August 22, 2005

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EXECUTIVE SUMMARY

National Starch seeks approval for the use of RS4-fibre* (*phosphated distarch phosphate), specifically made from high amylose maize starch, for use as a novel food ingredient in Europe. Approval is sought under Regulation (EC) No 258/97 of the European Parliament and of the Council of 27th January 1997 concerning novel foods and novel food ingredients (hereafter referred to as EC 258/97), and accordingly, this submission has been prepared pursuant to the Commission Recommendation of 29 July 1997 concerning the scientific aspects and the presentation of information necessary to support applications for the placing on the market of novel foods and novel food ingredients. RS4-fibre* will be considered under category (c), pertaining to "foods and food ingredients with a new or intentionally modified primary molecular structure".

Whilst RS4 fibre* as E1413 is currently approved as a "modified starch" food additive (meeting the EU food additive purity criteria), use levels and therefore intake quantities and patterns of the target populations will be significantly higher. The proposed food uses requested include biscuits (sweet), crackers, cakes and muffins, pasta, pizza dough, ready-to-eat breakfast cereals, tortillas, white bread, and pretzels at use levels of 12, 10, 20, 20, 20, 20, 20, 20., and 35.00 g/100 g of food, respectively. These use levels may result in theoretical worst-case mean and 97.5th percentile intakes of 32.4 and 72.2 g/person/day for RS4 fibre*, respectively, and 129.5 and 288.7 mg/person/day of residual (bound) phosphorous, respectively for all users. Considering the accuracy nature of the survey data and methodology used to calculate such intakes, these should be considered conservative (over-) estimations.

RS4 fibre* assays as minimum 70% fibre (resistant starch) by the AOAC 991.43 test method and will be labelled to this effect on finished food products. Consequently its caloric value of 0.464 kcal/g reflects a combination of digestible carbohydrate and fermentation of non-digestible carbohydrate by the bacteria of the lower gastrointestinal tract.

A full battery of toxicological studies have shown that RS4 fibre* has low toxicity at and good gastrointestinal tolerability as high levels in the diet for both the RS4 fibre itself and the resulting consumption of residual (bound) phosphorous. A human clinical study has shown no adverse effects at levels of 60g phosphated distarch phosphate (RS4 fibre) per day which is in the range of predicted realistic intake.

ADMINISTRATIVE DATA

Name and Address of Applicants/Manufacturers

The application (hereinafter referred to as National Starch) is submitted by:

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GENERAL DESCRIPTION

RS4-fibre (Modified Starch, Phosphated distarch phosphate) (hereafter referred to as RS4-fibre*) is a modified food starch refined from high amylose maize. According to Directive 95/2/EC, article 1,3 (q) modified starches used in food additive applications are defined 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." The Food Additives and Contaminants Committee defines a modified starch as, "…any product obtained from starch, the preparation of which has resulted in the modification of one or more of the properties of the starch from which it was prepared, but does not include malt extract or glucose syrup." The modification of starch with the aforementioned treatments results in the cross-linking and esterification of the starch chains with the overall extent of modification being small and the residual phosphate occurring in the range of approximately 0.4% according to JECFA (1972b) and the EU Purity Criteria, 2000/63/EC for food additives.

The EU definition for phosphated distarch phosphate (E1413) is defined as "starch having undergone a combination of treatments as described for monostarch phosphate and distarch phosphate" according to *Commission Directive 2000/63/EC of 5 October 2000 amending Directive 96/77/EC laying down specific purity criteria on food additives other than colours and sweeteners* (OJ 30.10.200 L277). Furthermore, the directive defines distarch phosphate as "starch cross-linked with sodium trimetaphosphate or phosphorus oxychloride", and

monostarch phosphate is defined as "starch esterified with ortho-phosphoric acid, or sodium or potassium ortho-phosphate or sodium tripolyphosphate". RS4-fibre is produced using the reagents listed within the purity criteria. It therefore meets the existing specification for modified starch food additive E1413.

These modified starches are currently used as freeze-thaw-stable thickeners. Cross-linked and stabilised starches improve the product quality and shelf-life stability of foods. These modified starches offer greater process stability and low temperature storage stability than their parent native starches and consequently can be used at a lower dosage rate. Typical products include soups, sauces, frozen gravies and pie fillings at a general usage level of between 2 to 5%.

RS4-fibre is a chemically modified starch which is categorised as a resistant starch, type 4. It analyses as being rich in dietary fibre enhancing its nutritional properties.

Although the term 'resistant starch' is not defined by any government agency (Goldring 2004), a group of scientists funded by the European Union (EU) in a concerted action known as EURESTA (FLAIR Concerted Action no. 11 *Physiological Implications of the Consumption of Resistant Starch in Man*) defined RS as the 'total amount of starch, and the products of starch degradation that resists digestion in the small intestine of healthy people' (Asp 1992).

Resistant starch (RS) has been classified into four general subtypes called RS1–RS4 (Englyst *et al*. 1992; Brown *et al*. 1995). RS1 is the term given to RS where the starch is physically inaccessible to digestion, *e.g.* due to the presence of intact cell walls in grains, seeds or tubers. RS2 describes native starch granules that are protected from digestion by the conformation or structure of the starch granule as in raw potatoes and green bananas. A particular type of RS2 is unique as it retains its structure and resistance even during the processing and preparation of many foods; this RS2 is called high-amylose maize starch. RS3 refers to non-granular starch-derived materials that resist digestion. RS3 forms are generally formed during the retrogradation of starch granules. Some examples of RS3 are cooked and cooled potatoes and cornflakes. RS4 describes a group of starches that have been chemically modified and include starches which have been etherised, esterified or cross-bonded with chemicals in such a manner as to decrease their digestibility. RS4 may be further subdivided into four subcategories according to their solubility in water and the experimental methods by which they can be analysed (Brown 2004).

National Starch intends to include RS4-fibre* as a resistant starch in foods to increase the dietary fibre content in various food products, in particular low moisture foods such as bread and bakery products, breakfast cereals, pasta and noodles, snacks, and breading. In these products it can be used to replace in part of the digestible carbohydrate in the diet, e.g. flour.

National Starch proposes to market RS4-fibre*, specifically made from high amylose maize starch, for use as a novel food ingredient in Europe. Approval is sought under Regulation (EC) No 258/97 of the European Parliament and of the Council of 27th January 1997 concerning novel foods and novel food ingredients (hereafter referred to as EC 258/97), and accordingly, this submission has been prepared pursuant to the Commission Recommendation of 29 July 1997 concerning the scientific aspects and the presentation of information necessary to support applications for the placing on the market of novel foods and novel food ingredients (hereafter referred to as the Commission Recommendation of 1997).

Article 1(2) of EC 258/97 states that the regulation "…shall apply to 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 one of 6 categories of novel foods and novel food ingredients. RS4-fibre* will be considered under category I, pertaining to "foods and food ingredients with a new or intentionally modified primary molecular structure".

Section 4 of the Commission Recommendation of 1997 outlines recommendations made by the Scientific Committee for Food (SCF) pertaining to the "Scientific Classification of Novel Foods for the Assessment of Wholesomeness", which facilitates the safety and nutritional evaluation of a given novel food/food ingredient. Of the six classes identified, RS4-fibre* would be classified as a Class 2 novel food (Complex NF from non-GM source), since it is produced by conventional methods (*i.e.*, without the use of genetic modification) as a complex. RS4-fibre* is further classified under sub-class 2.1 "the source of the NF has a history of food use in the Community" of the SCF categorization through the previous use of both starch *per se* and the use of the food additive E1413 phosphated distarch phosphate as a modified starch for technological purposes (*e.g.*, thickening). As a generally permitted food additive, under Directive 95/2/EC as amended, phosphated distarch phosphate (E1413) can be added to all foodstuffs, unless otherwise stated, to *quantum satis*, maximum level not specified, in accordance with good manufacturing practice at a level not higher than it is necessary to achieve the intended purpose.

IDENTIFICATION OF ESSENTIAL INFORMATION REQUIREMENTS

The structured schemes outlined for the assessment of a class 2.1 novel food ingredient, such as RS4-fibre*, are listed below and discussed in detail in subsequent sections (Sections I through XIII).

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 Exposures to the Novel Food or Its Source

XI. Nutritional Information on the Novel Food

XII. Microbiological Information on the Novel Food

XIII. Toxicological Information on the Novel Food

For each category (I through XIII), structured schemes have been developed by the SCF, which consist of a decision-tree-like set of questions designed to elicit sufficient data for a comprehensive safety and nutritional evaluation of the novel food. As outlined below in Sections I through XIII, the required questions are identified and subsequently addressed with the appropriate data.

I SPECIFICATIONS OF THE NOVEL FOOD

Based on the SCF guidelines, the following questions must be answered in the affirmative to ensure sufficient information pertaining to the specifications of the novel food:

- "…is appropriate analytical information available on potentially toxic inherent constituents, external contaminants and nutrients?"
- "Is the information representative of the novel food when produced on a commercial scale?"
- "Is there an appropriate specification (including species, taxon *etc*. for living organisms) to ensure that the novel food marketed is the same as that evaluated?"

These questions have been addressed collectively in Sections 1.1 through 1.8.

I.1 Common or Usual Name

RS4-fibre*

I.2 Chemical Names

Phosphated distarch phosphate

I.3 Trade Names

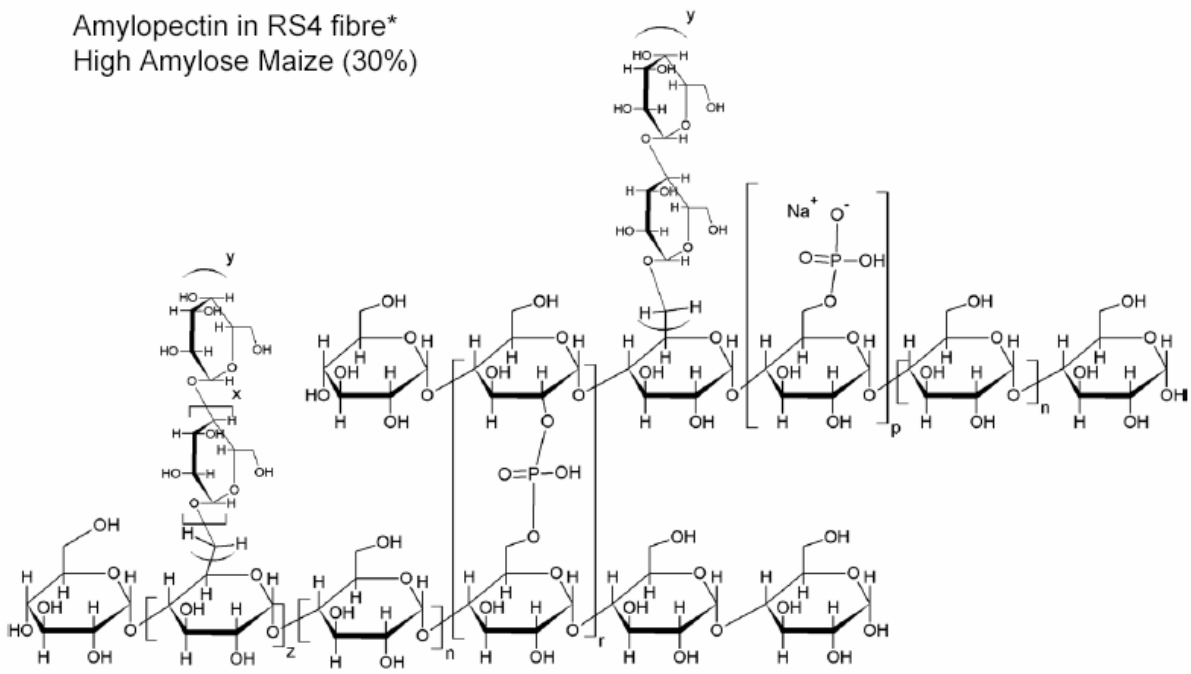
RS4-fibre modified starch (phosphated distarch phosphate); RS4 phosphated distarch phosphate; NOVELOSE® 480HA

I.4 Chemical Abstract Service (CAS) Number

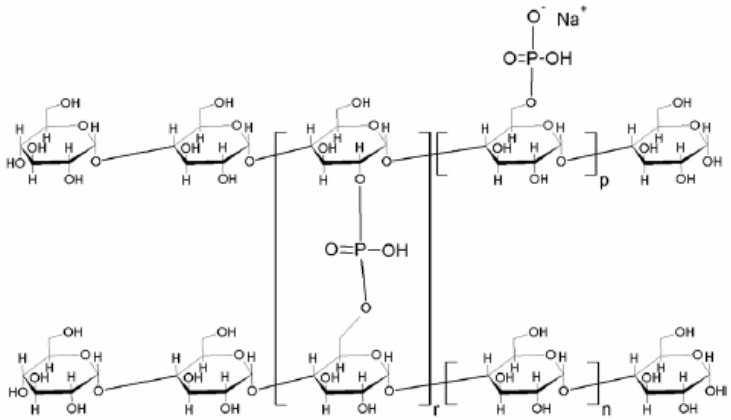
The CAS Number for phosphated distarch phosphate is 11120-02-8.

I.5 Chemical Structure

Starch consists of two polymers of glucose, amylopectin (highly branched) and amylose (almost linear). Most commercial sources of starch are only 17 to 25% amylose with the balance being amylopectin (Thomas *et al* 1999).



Amylose RS4 fibre*
in High Amylose Maize (70%)



I.6 Molecular Formula and Weight

Phosphated distarch phosphate:

$(C_6H_{10}O_5)_n\,[(C_6H_9O_5)_2PHO_2]_x[(C_6H_9O_5)PH_2O_3]_y$

Where; n = the number of glucose units linked together

x and y = the degree of substitution

Molecular weights:

Amylopectin 50,000,000

Amylose 200,000 (before cross-linking)

I.7 Chemical and Physical Properties

RS4-fibre* is a white or nearly white in colour and takes the form of a powder.

I.8 Product Specifications and Analysis

I.8.1 Product Specifications

The purity criteria for E1413 phosphated distarch phosphate when used as a food additive is laid out in the Official European Journal (Directive 2000/63/00), the Food and Chemical Codex (FCC, 2003) and have been summarized in Table I.8.1-1. However, National Starch has adopted additional specifications for RS4-fibre* in accordance with the Association of

Official of Analytical Chemists (AOAC) and the CML (National Starch Control Methods Laboratory). RS4-fibre* is identified by microscopic observation.

Table I.8.1-1 Chemical and Physical Specifications for RS4-fibre*

Parameter	Specification[1]	Test Method
General Specifications		
Appearance	Free flowing fine white powder	Visual inspection
Residual (bound) phosphorus	Not more than 0.4% (as phosphorus) "high amylose maize starch" as source	JECFA (2001)
Loss on drying (moisture content)	10 to 14%[2]	CML 116A[3]
Arsenic	Not more than 1 mg/kg	AOAC 985.01, 990.08, 984.27
Lead	Not more than 2 mg/kg	AOAC 985.01, 990.08, 984.27
Mercury	Not more than 0.1 mg/kg	AOAC 977.15
PH	4.5 to 7.5	CML 100A:20[4]
Sulphur dioxide	Not more than 10 mg/kg (dry basis)	CML 136A/M
Nutritional Data – Typical Values		
Carbohydrate	7.0% to 14%	Calculation
Starch[5]	7.0% to 14%	Calculation
Sugar[5]	0%	Calculation
Protein	0.8%	CML 117A, Kjeldahl
Fat	0.8%	AOAC 996.06
Saturated[6]	0.35%	AOAC 996.06
Cholesterol[6]	None detected	AOAC 920.39, 983.23, 933.05
Energy (caloric value)[7]	0.464 kcal/g (1.962 kJ/g)	Calculation
Total dietary Fibre	Minimum 70% ('as is', as packed)	AOAC 991.43
Microbiological Specifications		
Total viable count (TVC)	Not more than 10,000 cfu/g	CML261
Yeasts	Not more than 200 cfu/g	CML268
Moulds	Not more than 200 cfu/g	CML268
Escherichia coli	Absent/g	CML263
Salmonella	Absent/25 g	CML264

Adapted from the Official Journal of the European Union Commission Directive 2000/63/EC
[1] All values expressed on an 'as is', as packed basis
[2] Official European Journal (Directive 2000/63/00) specifies no more than 15.0% for cereal starch as the source (*i.e.*, high amylose maize starch)
[3] Analysis conducted for 4 hours at 130°C
[4] 10% aqueous suspension
[5] Percentage of carbohydrate content
[6] Percentage of fat content (*i.e.*, 0.35% of the 0.8% of fat is composed of saturated fat)
[7] Calculated in accordance with 90/496/EEC

I.8.2 Product Analysis

The product specifications for RS4-fibre* have been examined in 3 batches of manufactured product, the results of which are presented in Table I.8.2-1.

Table IX.I-1 Summary of the Individual Proposed Food-Uses and Use-Levels for RS4-fibre* and the Corresponding Use-Levels for Phosphorus in the UK

Analysis	Specifications	Lot Number				
		Batch 1	Batch 2	Batch 3	Batch 4	Batch 5
General Specifications						
Appearance	White powder	Yes	Yes	Yes	Yes	Yes
Residual (bound) phosphate (% as phosphorus)	≤0.4	0.365	0.354	0.358	.233	.258
Loss on drying (%)	10 to 14	12.1	11.7	12.0	13.2	10.9
Arsenic (mg/kg)	< 1	<0.1	<0.1	<0.1	<0.1	<0.1
Lead (mg/kg)	< 2	<0.1	<0.1	<0.1	<0.1	<0.1
Mercury (mg/kg)	< 0.1	<0.02	<0.02	<0.02	<0.02	<0.02
PH	4.5 to 7.5	5.6	5.6	5.8	6.1	5.9
Sulphur dioxide (mg/kg)	<10	<10	<10	<10	<10	<10
Nutritional Composition – Typical Values						
Carbohydrate (%)		14.0	7.0	9.0	9.0	8.0
Starch[4] (%)		14.0	7.0	9.0	9.0	8.0
Sugar[4] (%)		0	0	0	0	0
Protein (%)		<0.625	<0.625	<0.625	<0.625	<0.625
Fat (%)		0.99	1.0	0.96	1.0	0.93
Saturated (%)		0.42	0.44	0.43	0.46	0.40
Cholesterol (mg/100g)		<1.0	<1.0	<1.0	<1.0	<1.0
Total Dietary Fibre (%)('as is', as packed)		72	79.5	77	77	79
Microbiological Specifications						
Total viable count (TVC) (cfu/g)	< 10,000	< 10,000	< 10,000	< 10,000	< 10,000	< 10,000
Yeasts (cfu/g)	< 200	<10	<10	70	<10	<10
Moulds (cfu/g)	< 200	<10	<10	<10	<10	<10
Escherichia coli	Absent/g	<10	<10	<10	<10	<10
Salmonella	Absent/25 g	Negative	Negative	Negative	Negative	Negative

Residual (bound) phosphorus is determined using the JECFA method described in the monograph for Modified Starch [*Prepared at the 57th JECFA (2001), superseding specifications prepared at the 35th JECFA (1989), published in FNP 49 (1990) and in FNP 52 add 5(1997)*]. In this procedure the starch sample (*i.e.*, 20 to 25 g in a 250 mL beaker) is first suspended in a 7 to 3 methanol-water mixture (*i.e.*, 200 mL), dispersed, and agitated mechanically (*i.e.*, approximately for 15 minutes). The starch is recovered *via* vacuum filtration (*i.e.*, in a 150 mL medium-porosity fritted-glass or Buchner funnel). The wet cake is washed with the methanol-water mixture (*i.e.*, 200 mL), then the wet cake is reslurried in the solvent and washed a second time in the same manner. Therefore, the phosphorus content reported as residual is primarily bound to the starch polymer and may contain traces of un-reacted phosphorus.

In addition to the above specifications, microbiological testing was performed on a number of batches of United States (US) manufactured product. The batches were analysed for total viable count (total plate count) and the presence of yeast, mould, *Escherichia coli*, and *Salmonella typhimurium.* The total viable count was reported to range from 10 to 10,000 cfu/g, moulds were all less than 10 cfu/g, and was present at levels ≤10 cfu/g in all batches tested. Furthermore, all batches were reported to test negative for the presence of *E. coli* and *S. typhimurium*. All microbiological parameters tested were within the specified limits. The results of the batch analyses are provided in Annex A.

Similarly, numerous manufactured batches were tested for the presence of mycotoxins including aflatoxin B1, B2, G1, and G2, deoxynivalenol, zearalenone, fumonisin B1, B2, and B3, and ochratoxin A. The detection limits for all of the mycotoxins assayed were 0.01 ppb for all aflatoxins (*i.e.*, B1, B2, G1, and G2); 0.1 ppm for deoxynivalenol, fumonisins (*i.e.*, B1, B2, and B3); 50 ppb for zeralone; and 0.05 ppb for ochratoxin A. The mycotoxins were not detected above the detection limit in the batches assayed. Furthermore, lead and nitrates with detection limits of 0.1 and 10 ppm, respectively, also were not detected above their respective detection limits in the batches assayed. The results of the analysis are provided in Annex B.

I.8.3 Product/Raw Material Food Safety Analysis

In addition to analyses performed on the RS4-Fibre*, samples of finished product and the raw starting material, unmodified high amylose maize, typical to the site of manufacture are examined on a quarterly survey basis for pesticide residues (*e.g.*, organochlorine, organophosphorous, pyrethroid, and miscellaneous pesticides), heavy metals, mycotoxins, nitrosamines, and microbiological contamination. Batch EEX 6612 is representative of the type and extent of the analyses carried out. Typically, no pesticide residues were reported above the limit of detection in the batches tested. Similarly, toxic metals/minerals assayed in batch EEX 6612and were reported to be detected at levels of less than 0.001 ppm for lead, mercury, cadmium, and thallium; <0.01 for arsenic; and <0.1 for bromate. Mycotoxin

analysis demonstrated levels of aflatoxins B1, B2, G1, and G2 at <0.05 ppb each; zeralone at <1 ppb; fumonisin B1 and B2 at <0.1 ppb each; ochratoxin A and B at <0.1 ppb each; and deoxynivalenol at <5 ppb. Total nitrosamine, nitrite, and nitrate levels were less than 5, 5, and 10 ppb, respectively. Microbiological analysis of batch EEX 6612 revealed the absence of *Salmonella* in 25 g. The results of the analysis of batch EEX 6612provided in Annex C illustrates the typical levels of pesticide residues, nitrosamines, heavy metals, mycotoxins, and microbiological content of a finished product manufactured by National Starch and is representative of the routine food safety analysis.

II EFFECT OF THE PRODUCTION PROCESS APPLIED TO THE NOVEL FOOD

Based on the SCF guidelines, the following questions must be addressed to ensure sufficient information pertaining to the effect of the production process applied to the novel food:

- "Does the novel food undergo a production process?"
- "Is there a history of use of the production process for the food?"

These questions have been addressed collectively in Sections II.1 through II.5.

II.1 Novelty of the Process

Whilst the production process used to make RS4-fibre*, is not in itself a "novel" method in terms of food production *per se*, specific raw materials are used for its manufacture as detailed below.

II.2 Raw Materials and Chemicals Specifications

II.2.1 High Amylose Maize

Some varieties of maize are homozygous for the amylose extender or "ae1" gene, the presence of which results in a starch with 50 to 80% amylose. High "amylomaize" varieties have very high gelatinisation temperatures (154 to 171°C), which means that the starch granules are resistant to gelatinisation at normal cooking temperatures (Brown *et al.*, 2001). Gelatinisation of starch granules usually occurs when food is cooked in water at boiling temperatures. The granules rupture, allowing access to digestive enzymes when the food is consumed. If the granules do not rupture, the starch within them is less susceptible to digestion by amylase in the human small intestine, and is therefore resistant. Since the granules of high amylomaize varieties do not rupture at usual boiling temperatures, they therefore contain high levels of resistant starch. [redacted]

[redacted] The seed and crop are tested for non-GM along the entire grain supply chain from seed production to final delivery into the plant [redacted]

II.2.2 Chemical Reagents

RS4-fibre*, also known commonly as a form of phosphated distarch phosphate, is formed through esterification and cross-linking of unmodified high amylose maize starch molecules with [redacted] which also are used for the formation of monostarch or distarch phosphate, respectively (Weedon *et al.*, 1980; JECFA, 2001).

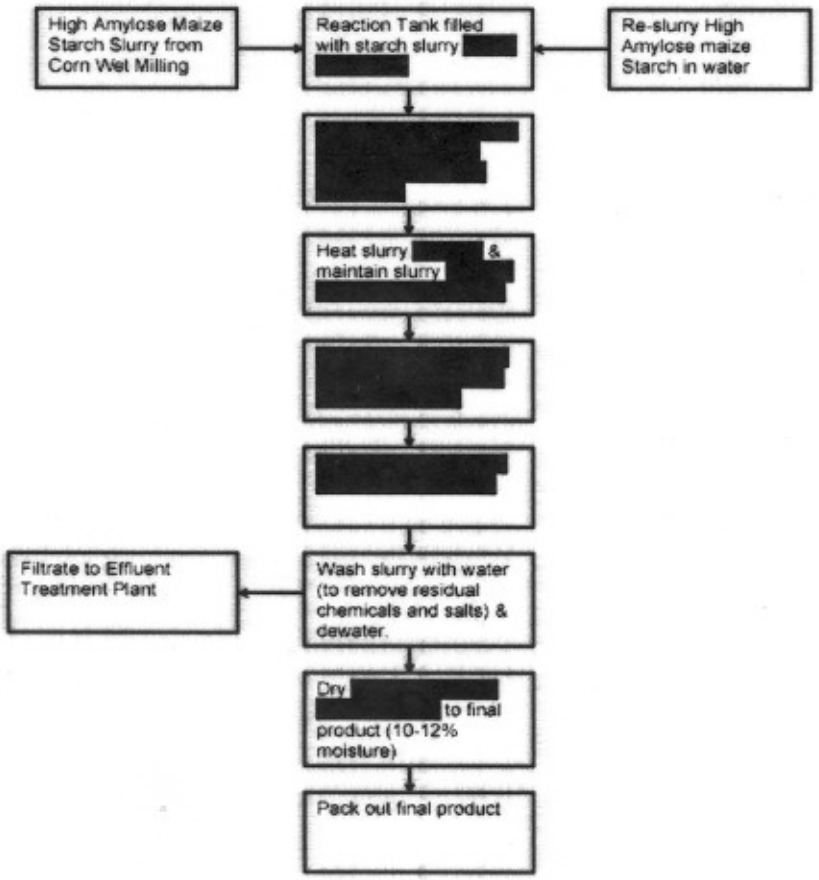
[redacted]

II.3 Description of the Manufacturing Process

RS4-fibre* is produced through [redacted] a combination of treatments used for preparing monostarch phosphate and distarch phosphate, respectively.

A schematic overview of the manufacturing process is given in Figure II.3-I. The maize grain is taken onto plant and is then milled via a corn steeping process into a slurry form (referred to as starch slurry) to facilitate manufacture. The diagram specifies that the starting material is high-amylose maize starch slurry derived from wet milling of the grain and a re-slurry of high corn starch in water.

Figure II.3-1 Schematic Overview of the Manufacturing Process for RS4-fibre*



II.4 Potential Impurities Resulting from the Production Process

This issue is addressed with Section I.8.2 above.

II.5 Stability of RS4-fibre*

The shelf life for modified starches of the type RS4-fibre* was in the past considered to be indefinite when stored correctly in sealed containers. However, in 1997 the European Starch Industry (Association des Amidonneries de Cereales de L'ue - AAC) agreed to an industry standard of an advisory best before date of 24 months for all food starches. National Starch, therefore, typically give a shelf life of 720 days for food starches. This is viewed to be a very conservative estimate. National Starch would not expect any significant chemical changes in the RS4-fibre* product over that time period.

II.6 Hazard Analysis and Critical Control Point (HACCP)

National Starch has developed HACCP procedures for each step of the production process form purchase of the raw materials through the shipping of the finished goods. At each step of the process potential hazards have been identified and control measures have been put into place to maintain the quality of the product and the safety of the manufacturing personnel. A summary table of the HACCP areas and solutions is provided in Annex E [CONFIDENTIAL].

III HISTORY OF THE ORGANISM USED AS THE SOURCE

Based on the SCF guidelines, the following questions must be addressed to ensure sufficient information pertaining to the history of the source organism:

- "Is the novel food obtained from a biological source (*i.e.*, a plant, animal or microorganism)?"
- "Has the organism been used as the source of the NF been derived using GM?"
- Is the source of the organism characterised?"
- "Is there information to show that the source organism and/or foods obtained from it are not detrimental to human health?"

III.1 Origin of RS4-fibre* Source

The source of RS4-fibre* is unmodified, high amylose maize starch. The EU specifications for phosphated distarch phosphate indicate that traditional unmodified starches are used to prepare phosphated distarch phosphate through a combination of the treatments used to prepare monostarch phosphate and distarch phosphate. The raw materials and processing methods to produce RS4-fibre* are discussed in detail within section II above.

The term "resistant starch" has not been defined by governmental agency (Goldring, 2004); however, a group of European Union (EU)-funded scientists as part of a concerted action known as EURESTA have defined resistant starch as the "total amount of starch, and the products of starch degradation that resists digestion in the small intestine of healthy people" (Asp, 1992). The forms of resistant starch have been divided into 4 general subtypes, RS1 through RS4 based on their reason to resist digestion (Brown, 2004). RS4-fibre* manufactured by National Starch is produced from high amylose maize (a natural hybrid of regular maize) starch, which is a unique form of starch as it retains its granular conformation, as well as its resistant starch and dietary fibre content during many processes used in the manufacturing of consumer food products. Brown (1995) reported that a corresponding elevation in the recorded dietary fibre and resistant starch content is seen with increases in the amount of amylose in the starch granule. RS4-fibre*, also known commonly as a form of phosphated distarch phosphate, is a RS4-type resistant starch prepared from high amylose maize using a combination of treatments to reduce its digestibility.

Furthermore, as there are 4 subtypes of resistant starches, RS4-type resistant starches have been further subdivided into 4 subcategories based on the ability of the RS4 subtypes to be analysed using *in vitro* techniques for dietary fibre (Prosky *et al.*, 1985, 1994) and resistant starch (McCleary *et al.*, 2002a,b), or resistant starch alone (Brown, 2004). The categorization of the subtypes of RS4 starches is also based on whether the subtype is soluble or insoluble in water. RS4-type resistant starches have been used in Australia in commercial food products with a dietary fibre content of 2.9 to 5.6% since 1994 and in Japan in commercial food products with a dietary fibre content of 2 to 6% since 1995. In addition, commercial products containing RS4-type resistant starch as a dietary fibre source are now widely available on the US and Canadian markets (see Annex H).

Resistant starches have been reported to possess a wide range of physiologic effects such as acting as a prebiotic for the microflora in the human colon thereby providing the host with the various metabolites produced by the bacteria (*e.g.*, short-chain fatty acids, carbon dioxide, methane, and hydrogen) (Brown, 2004; Nugent, 2005). Other general physiological effects include reducing the glycemic response, reducing energy (*i.e.*, fewer calories absorbed), improving bowel health, culture protagonist, increase micronutrient absorption, possible protection again bowel cancer, and possible synergistic effects with other dietary components

(*e.g.*, fibres, proteins, lipids) (Brown, 2004; Nugent, 2005). Resistant starch (RS) is a major component of intact whole grains and many raw vegetables. In certain raw foods the RS level can be as high as 70% (Englyst, 1992), but when these foods are cooked the RS level decreases significantly to as low as <1% (depending on the extent of food processing). High amylose RS4-fibre* is unique in that it maintains its RS and dietary fibre composition even when it has been cooked or processed during food manufacturing.

National Starch intends to include RS4-fibre*, an economical fibre source, as a resistant starch that will be widely available for use in processed foods to increase their dietary fibre content bring benefit to the consumer by increasing the fibre consumption in the diet. An important advantage of RS4-fibre* is that it is both easy to use and nice to eat. This enables food manufacturers to cost-effectively incorporate it into everyday foods.

IX ANTICIPATED INTAKE/EXTENT OF USE OF NOVEL FOOD

Based on the SCF guidelines, the following questions must be addressed to ensure sufficient information pertaining to the effect of the production process applied to the novel food:

- "Is there information on the anticipated uses of the novel food based on its properties?"
- "Is there information to show anticipated intakes for groups predicted to be at risk?"
- "Will introduction of the novel food be restricted geographically?"
- "Will the novel food replace other foods in the diet?"
- "Are any of the replaced foods significant nutritional sources?"
- "Does the probable level of substitution have a nutritional significance for any population groups?"

These questions have been addressed collectively in Sections IX.1 through IX.3.

IX.1 Intended Uses in Food

RS4-fibre* is intended for use in low moisture conventional foods to increase the dietary fibre content of foods and act as a resistant fibre. The individual proposed use-levels for RS4-fibre* and phosphorus employed in the current intake analysis are summarized in Table IX.1-1. Food codes representative of each proposed food-use were chosen from the MAFF food code list associated with each food consumption survey and grouped in food-use categories according to the food type, main and subsidiary food group classifications detailed within the NDNS reports (UKDA, 1991, 1995, 2001). All food codes included in the current intake assessment are listed in Annex F. A given food code may not be associated with all 3 surveys; as with each new survey the food code list has been updated to reflect the availability of new foods and the discontinuation of certain obsolete codes.

Table IX.I-1 Summary of the Individual Proposed Food-Uses and Use-Levels for RS4-fibre* and the Corresponding Use-Levels for Phosphorus in the UK

Food Category	Proposed Food-Uses	RS4-fibre*		Phosphorus	
		Use-Level (g/100 g food)	Maximum Use-Level (%)	Use-Level (mg/100 g food)*	Use-Level (%)
Cereals and Cereal Products (including bakery products)	Biscuits (sweet)	12.00	12.00	48	0.048
	Crackers	10.00	10.00	40	0.040
	Cakes and Muffins	20.00	20.00	80	0.080
	Pasta	20.00	20.00	80	0.080
	Pizza Dough	20.00	20.00	80	0.080
	Ready-to-Eat Breakfast Cereals	20.00	20.00	80	0.080
	Tortillas	20.00	20.00	80	0.080
	Bread products made with white flour	20.00	20.00	80	0.080
Crisps and Savoury Snacks	Pretzels	35.00	35.00	140	0.14

RS4-fibre* contains 0.4% phosphorus

National Starch intends to include RS4-fibre* as a resistant starch in foods to increase the dietary fibre content in various food products, in particular low moisture foods such as bread and bakery products, breakfast cereals, pasta and noodles, snacks, and breading. In these products it can be used to replace in part of the digestible carbohydrate in the diet, e.g. flour.

IX.2 Estimated Consumption of RS4-fibre* from Proposed Food-Uses in the EU

IX.2.1 Estimated Daily RS4-fibre* Intake from All Proposed Food-Uses

Estimates for the intake of RS4-fibre* and phosphorus in the E.U. were based on the proposed use-levels for RS4-fibre* in Table IX.1-1 and food consumption data collected as part of the United Kingdom (U.K.) Food Standards Agency's, Dietary Survey Programme (DSP). The main component of the DSP is the U.K. National Diet and Nutrition Survey (NDNS) programme commissioned jointly in 1992 by the Ministry of Agriculture, Fisheries and Food (MAFF) and the Department of Health, and transferred to the Food Standards Agency on its inception in April 2000. The NDNS programme consists of four different surveys for specific age groups, conducted approximately every 3 years in succession. Separate survey data are available from the U.K. Data Archive (UKDA) for The NDNS: Adults Aged 16 to 64 years collected in 2000-2001 (NDNS 2000-2001) (Office for National Statistics, 2005), the National Diet, Nutrition and Dental Survey of Children Aged 1½ to 4½ Years, 1992-1993 (NDNS, 1992-1993) (UKDA, 1995), the National Diet and Nutrition Survey: Young People aged 4 to 18 Years (NDNS, 1997) (UKDA, 2001), and the National Diet and Nutrition Survey: People Aged 65 Years and Over, 1994-1995; however, only the former three surveys were used to generate estimates in the current intake analysis. Combined, these surveys provide the most up-to-date data for evaluating food-use, food-consumption patterns, and nutritional status in the U.K., containing 4- or 7-day weighed food records for individuals selected using a stratified multi-stage random probability design, with sampling of private households throughout Great Britain using postal sectors (UKDA, 1995, 2001) or local authority wards (UKDA, 1991) as the primary sampling unit.

NDNS data were collected from individuals and households *via* 4- (children, aged 1½ to 4½) or 7-day (young people, aged 4 to 18 and adults, aged 16 to 64) weighed dietary intake records throughout all 4 seasons of the year (4 fieldwork waves of 3 months duration), in order to address variability in eating behaviours due to seasonality. Dietary data were recorded by survey respondents, or in the case of the children's survey, by parents or guardians, for the duration of the survey period. The adult NDNS 2000-2001 contains 7-day weighed dietary records for 1,724 individuals aged 16 to 64 who were not pregnant or breastfeeding, while, NDNS 1992-1993 contributes 4-day data from an additional 1,592 children 1½ to 4½ years of age. NDNS 1997 adds 7-day records for approximately 1,700 youth aged 4 to 18 (Office for National Statistics, 2005; UKDA, 1995, 2001). The initial postal and interview sifts to identify eligible children, youth, or adults, respectively, for the surveys identified 93%, 92%, and 65% eligibility; the maximum response rate (individuals agreeing to the initial dietary interview) from the eligible sample selected for participation in the survey were, 88%, 80%, and 61%, respectively, while only 81%, 64%, and 47% of

surveyed individuals completed a full dietary record (Office for National Statistics, 2005; Gregory et al., 1995; UKDA, 2001).

In addition to collecting information on the types and quantities of foods being consumed, the NDNS programme collects physiological, anthropometric and demographic information from individual survey participants, such as sex, age, measured height and weight (by the interviewer), blood analytes, and other variables useful in characterizing consumption. The inclusion of this information allows for further assessment of food intake based on consumption by specific population groups of interest within the total surveyed samples. Sample weights were developed and incorporated with the youth survey (NDNS, 1997) to compensate for the potential under-representation of intakes from specific population groups as a result of sample variability due to differential sampling probabilities and differential non-response rates, particularly the lower response obtained from males, aged 15 to 18 years (UKDA, 2001).

To facilitate comparison with the adult and youth 7-day dietary survey data, dietary data from the children's survey (4-day data) was weighted to 7 days, based on the assumption that intake patterns on non-recording weekdays were similar to dietary intakes on recorded weekdays; the 2 weekend days were not re-weighted. Accordingly, all food and drink consumed on the 2 recorded weekdays were averaged to give a daily intake value, which was multiplied by 5 to approximate intakes for all weekdays. These values were then combined with consumption data from weekend dietary records. Full details of the weighting method applied are provided in Appendix J of the report on the children's diet and nutrition survey (Gregory *et al.*, 1995). It is also noted in this report that short-term surveys, such as the 4-day children's survey, may overestimate consumption of food products that are consumed relatively infrequently, particularly when weighted to 7 days.

Consumption data from individual dietary records, detailing food items ingested by each survey participant on each of the survey days, were collated by computer and used to generate estimates for the intakes of RS4-fibre* and phosphorus by the U.K. population. Estimates for the daily intakes of RS4-fibre* and phosphorus represent projected 7-day averages for each individual from Days 1 to 7 of NDNS data; these average amounts comprised the distribution from which mean and percentile intake estimates were produced. Mean and percentile estimates were generated using ratio estimation and nonparametric techniques, incorporating survey weights where appropriate (*i.e.* when using youth data to estimate intakes, as described in Section 2.1) in order to provide representative intakes for specific U.K. population groups. All-person intake refers to the estimated intake of RS4-fibre* and phosphorus averaged over all individuals surveyed regardless of whether they consumed food products in which RS4-fibre* is currently proposed for use, and therefore includes "zero" consumers [those who reported no intake of food products containing RS4-fibre* during the 7 survey days]. All-user intake refers to the estimated intakes of RS4-fibre*

and phosphorus by those individuals consuming food products in which the use of RS4-fibre* is under consideration, hence the 'all-user' designation. Individuals were considered users if they consumed 1 or more food products in which RS4-fibre* is proposed for use on one of the 7 survey days.

Calculations for the mean and high-level (97.5th percentile) all-person and all-user intakes, and percent consuming were performed for each of the individual proposed food-uses for RS4-fibre*. Similar calculations were used to determine the estimated total intake of RS4-fibre* and phosphorus from all proposed food-uses combined. In both cases, the per person and per kilogram body weight intakes were reported for the following population groups:

children, ages 1½ to 4½ ;
young people, ages 4 to 10;
female teenagers, ages 11 to 18;
male teenagers, ages 11 to 18;
female adults, ages 16 to 64;
male adults, ages 16 to 64.

The estimated total consumption of RS4-fibre* from all proposed food uses is summarized in Tables IX.2.1-1 on a daily g per person. A complete intake report is provided in Annex F.

As would be expected for a 7-day survey, the percentage of users was high among all age groups evaluated in the current intake assessment; greater than 97% of the population groups consisted of users of those food products in which RS4-fibre* is currently proposed for use, and the proportion of users remained essentially the same in adults relative to children and young people (Table IX.2.1-1). Young people and male teenagers had the greatest percentage of users at 99.6% and 99.5%, respectively, followed by children and female teenagers at 98.6% and 97.8%, respectively. Large user percentages within a population group typically lead to similar results for the all-person and all-user consumption estimates. Consequently, only the all-user intake results will be discussed in detail. Of the individual population groups, male teenagers were determined to have the greatest mean all-user intake of RS4-fibre, while male adults were determined to have the greatest 97.5th percentile all-user intakes of RS4-fibre* on an absolute basis with intakes of 32.4 g/person/day and 72.2 g/person/day, respectively, while children had the lowest intakes of 14.5 and 31.6 g/ person/day, respectively (Table IX.2.1-1). When assessed by sex, estimated daily RS4-fibre* intakes were lower in females relative to males.

Table IX.2.1-1 Summary of the Estimated Daily Intake of RS4-fibre* from All Proposed Food Categories in the U.K. by Population Group (NDNS Data)

Population Group	Age Group (Years)	% User	Actual # of Total Users	All-Person Consumption				All-Users Consumption			
				Mean (g)	Percentile (g)			Mean (g)	Percentile (g)		
					90	95	97.5		90	95	97.5
Children	1½ - 4½	98.6	1625	14.4	24.2	27.7	31.6	14.5	24.2	27.7	31.6
Young People	4-10	99.6	834	26.1	39.4	46.0	49.7	26.2	39.6	46.0	49.9
Female Teenager	11-18	97.8	436	26.0	40.7	46.5	54.2	26.1	40.7	46.6	54.2
Male Teenager	11-18	99.5	414	34.5	55.1	59.5	69.3	34.5	55.1	59.5	69.3
Female Adults	16-64	93.2	893	22.0	36.6	42.6	49.7	22.3	37.0	42.8	49.7
Male Adults	16-64	94.3	722	31.9	54.7	62.8	71.6	32.4	55.3	63.4	72.2

In addition to the estimated daily intake of RS4-fibre*, the estimated daily intake of phosphorous in relation to the intake of RS4-fibre* was determined based on residual (*i.e.*, bound) phosphorous content of 0.4% (refer to Table IX.I-1) with the intake of the individual population groups as total daily intake on an absolute basis (mg/person/day) summarized in Table's IX.2.1-3,. Of the individual population groups, male teenagers were determined to have the greatest mean all-user intakes of phosphorous on an absolute basis and male adults were determined to have the greatest 97.5th percentile all-user intake of phosphorous with values of 129.5 and 288.7 mg/person/day, respectively. Conversely, children had the lowest mean and 97.5th percentile all-user intakes of phosphorous from RS4-firbre with values of 58.0 and 126.4 mg/person/day, respectively (Table IX.2.1-3). When assessed by sex, estimated daily RS4-fibre* intakes were lower in females relative to males.

Table IX.2.1-3 Summary of the Estimated Daily Intake of Phosphorus from All Proposed Food Categories in the U.K. by Population Group (NDNS Data)

Population Group	Age Group (Years)	% User	Actual # of Total Users	All-Person Consumption				All-Users Consumption			
				Mean (mg)	Percentile (mg)			Mean (mg)	Percentile (mg)		
					90	95	97.5		90	95	97.5
Children	1½ - 4½	98.6	1625	57.8	96.7	110.7	126.4	58.0	96.7	110.7	126.4
Young People	4-10	99.6	834	104.4	157.6	184.2	198.7	104.6	158.5	184.2	199.5
Female Teenager	11-18	97.8	436	104.0	162.7	186.1	216.9	104.4	163.0	186.5	216.9
Male Teenager	11-18	99.5	414	138.0	220.2	237.8	277.2	138.0	220.2	237.8	277.2
Female Adults	16-64	93.2	893	88.1	146.2	170.4	198.7	89.3	148.0	171.2	198.7
Male Adults	16-64	94.3	722	127.4	218.9	251.4	286.3	129.5	221.1	253.7	288.7

It should be noted that the top level of exposure to RS4-fibre* is in adult males and amounts to 72.2 g/person/day (for all users). As RS4-fibre* is minimum 70% dietary fibre that equates to less than or approximately 60g of dietary fibre per day. The current fibre consumption is approximately 12 to 14 g/day in the UK. Guideline is to increase this to 24 g of fibre. It is unlikely that the introduction of RS4-fibre* will ever result in intakes of 60 g of fibre/day since this would necessitate the formulation of RS4-fibre* into all staple starchy foods. It is possible, however, that the, addition of RS4* fibre in place of some of the unmodified starch in the diet could make a significant contribution towards government objectives.

IX.2.2 Estimated Daily RS4-fibre* and Phosphorus Intake from Individual Food-Uses in the E.U.

Estimates for the all-user intakes of RS4-fibre* and phosphorus from each of the individual food-uses demonstrated that (Appendices A, B, C, and D of the Intake Report; see Annex F of the dossier) the highest mean and 97.5th percentile all-user intakes of RS4-fibre* were identified in male adults consuming white bread, at 18.13 and 47.23 g/person/day. For phosphorus, male adults consuming white bread also was demonstrated to have the highest mean and 97.5th percentile all-user intakes of phosphorus from RS4-fibre*, at 72.53 and 188.91 mg/person/day. .

IX.3 Food Product Labelling Information

The labelling of food products containing RS4-fibre* has been addressed in Section XI.2.

IX.4 Conclusion

Consumption data and information pertaining to the individual proposed food-uses for RS4-fibre* were used to estimate the all-person and all-user RS4-fibre* intakes of specific demographic groups in the U.K. population. This type of intake methodology is generally considered to be ‘worst case’ as a result of several conservative assumptions made in the consumption estimates. For example, it is often assumed that all food products within a food category contain the ingredient at the maximum specified level of use. In addition, it is well established that the length of a dietary survey affects the estimated consumption of individual users. Consequently, it can be argued that the predicted 97.5th percentile all-user consumption figures described above of 72.2 g/person/day and 288.7 mg/person per day RS4 fibre* and phosphorous respectively for teenage males are considerable over-estimations.

X INFOR0MATION FROM PREVIOUS HUMAN EXPOSURES TO THE NOVEL FOOD OR ITS SOURCE

Based on SCF guidelines, the following questions must be answered in the affirmative to ensure sufficient information pertaining to previous human exposure to the novel food:

- "Is there information from previous direct, indirect, intended or unintended human exposure to the novel food or its source which is relevant to the EU situation with respect to production, preparation, population, lifestyles and intakes?"

- "Is there information to demonstrate that exposure to the novel food is unlikely to give rise to mitochondrial, toxicological and/or allergenicity problems?"

These questions have been addressed collectively in Section 5.1 through 5.3.

X.1 Natural Occurrence of RS4-fibre* in the Diet

RS4-fibre* does not occur naturally in the human diet; however, unmodified starches are typically present in the diet of British adults comprising approximately 150 g of unmodified starch or 24% of their daily energy according to the Dietary and Nutritional Survey of British Adults (COMA, 1991).

X.1.1 Intake of RS4-fibre* in Europe and the Rest of the World

The approximate background intake of phosphated distarch phosphate, or rather RS4-fibre*, in the general population is 17 mg/kg body weight/day (FASEB, 1979). As a result of the lack of data available concerning the background intake of various modified starches, FASEB (1979) stated that it typically assumes a background intake of 17 mg/kg body weight/day for the purposes of safety evaluations.

RS4 fibre (phosphated distarch phosphate) is already being sold as a source of fibre outside the EU. We provide here as Annex H details of such products being sold in the US and Canada. The product "Fibresym RS4" was launched by MGP in autumn 2003. More information is available from the following weblinks:

http://www.mgpingredients.com/bakery/00_frame.htm;
http://www.aaccnet.org/meetings/2000/Abstracts/a00ma178.htm

X.1.2 Potential Toxicological Concerns

The potential toxicological effects of RS4-fibre* have been addressed in Section XIII.

X.1.3 Potential Allergenicity Concerns

The potential allergenicity of RS4-fibre* has been addressed and is covered in Section XIII.5. The product is considered free of gluten.

XI NUTRITIONAL INFORMATION ON THE NOVEL FOOD

Based on the SCF guidelines, the following question must be answered in the affirmative to ensure sufficient nutritional information pertaining to the novel food:

- "Is there information to show that the novel food is nutritionally equivalent to existing foods that it might replace in the diet?"

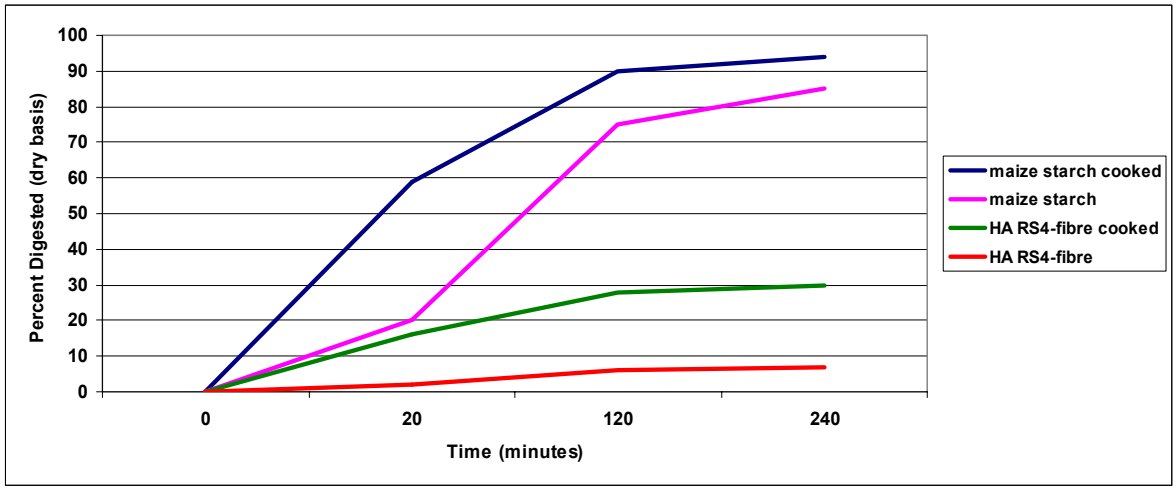
This question has been addressed in Sections XI.1.

XI.1 Nutritional Equivalence to Existing Foods

Phosphated distarch phosphate was demonstrated to be nutritionally equivalent to uncooked unmodified starch and distarch phosphate in a nutritional assay with rats (Kohn and Kay (1963b). Rats (10/group) were administered 1.0, 2.4, or 4.0 g of phosphated distarch phosphate, distarch phosphate, or unmodified starch, with an additional group fed a basal diet 5.0 g/day without supplemental starch for 10 days. The weight gain was reported to be similar between the treatment groups (phosphated distarch phosphate and distarch phosphate) and the reference supplement groups (white unmodified starch). See also Section XIII.2.1 on ADME.

Standard unmodified starch *(e.g.*, raw potato starch) is very rich in resistant starch in its uncooked state; however, when standard unmodified starch is cooked the starch gelatinises and the structure opens up and becomes very rapid to digest. For example, the resistant starch content of potatoes when boiled will drop to <1%. This is why food processing, whilst improving the safety and quality of various foods, can have a negative impact on the nutritional quality of the carbohydrates by making them very fast to digest more like sugar. Starch consists of two polymers of glucose, amylopectin (highly branched) and amylose (almost linear). Most commercial sources of starch are only 17 to 25% amylose with the balance being amylopectin (Thomas *et al* 1999). Due to it's almost linear and therefore compact structure, high amylose maize (>70% amylose) is able to withstand some food processing techniques and thereby retain its original physical structure. By further enhancing this effect wit h the RS4 process, the RS4-fibre* has a high dietary fibre level and is more able to withstand cooking and commercial food processing techniques. Figure XI.1-1 illustrates this property through the comparison of uncooked and cooked unmodified maize starch and high amylose RS4-fibre.

Figure XI.1-1 Englyst Digestion Evaluations of High Amylose RS4-fibre and Regular Maize



The above figure demonstrates that high amylose RS4-fibre* retains its a dietary fibre content whether eaten raw or cooked. Brown *et al.* (2003) examined the effect of processing (*i.e.*, cooking) on the digestibility (*i.e.*, the postprandial insulinemic and glycemic response) of carbohydrate meals composed of different amounts of amylose. Male Wistar rats were each fed a diet with an amylose content of 0, 270, 600, or 850 g/kg total starch, cooked or uncooked at different times throughout the study. The results of the study demonstrated that starches high in amylopectin (0 g amylose/kg total starch) are readily digestible; however, only a small amount of uncooked amylose starch (270 g amylose/kg total starch) is required to reduce postprandial insulin concentrations. Following cooking a higher proportion of amylose starch (*e.g.*, 600 or 850 g amylose/kg total starch) is required to elicit a significant reduction in the insulinemic response compared starches high in amylopectin (0 g amylose/kg total starch). Since RS4-fibre* retains its dietary fibre content following cooking it can be used in everyday processed foods to improve the carbohydrate digestion profile (*i.e.* physiologically) through a reduction in the postprandial glycemic response, thereby eliciting a response more similar to unprocessed/raw foods.

XI.2 Food Product Labelling Information

Food products containing phosphated distarch phosphate produced by National Starch from high amylose maize will be labelled as follows:

*RS4-fibre** - on the front label

** modified starch (phosphated distarch phosphate).* – in the ingredients listing

XI.2.1 The Nutritional Panel

Table XI.2.1-1 Nutrition Panel for RS4 fibre*

Nutritional Information	**Typical Values per 100 g "As is"**
Energy	196.2 kJ / 46.4 kcal
Protein	0.8 g
Carbohydrate of which sugars of which starch	9.0 g 0.0 g 9.0 g
Fat of which saturates	0.8 g Trace
Fibre (**)	Minimum 70 g
Sodium	8.5 mg

(**) Dietary Fibre content measured via AOAC methodology 991.43

The caloric value (*i.e.*, energy value) as determined by National Starch, will be listed as 0.464 kcal/g (1.962 kJ/g). Approximately 70 to 80% (a minimum of 70%) of the RS4-fibre* will be dietary fibre, which is resistant to degradation within the small intestine resulting in the majority of the non-digestible mass passing through the small intestine to the colon where it is metabolised and volatile free fatty acids are released and absorbed from the colon as energy.

RS4-fibre* (phosphated distarch phosphate), tests as min 70% Total Dietary Fibre (TDF) by AOAC 991.43 method (Annex G). It is classified as resistant starch type 4 (RS4). RS4 describes a group of starches that have been chemically modified in such a manner as to decrease their digestibility. The dietary fibre properties of RS4 have been reviewed by the AOAC (Brown, 2004) and the British Nutrition Foundation (Nugent, 2005).

Considerations of EVM phosphorus labelling recommendations

The Food Standards Agency (FSA) and industry have been working together to develop a labelling initiative to permit the use of phosphorus (as phosphate) levels above current use levels in food supplements, as well as above levels recommended by the EVM Committee. The FSA proposed the use of 2 advisory statements warning of possible side effects including gastrointestinal upset and bone effects. The EVM (2003) derived a guidance value of 250 mg of phosphorus/day based on mild gastrointestinal effects reported in postmenopausal women at a lowest observed adverse effect level of 750 mg/day, and adjusted (*i.e.*, divided by a factor of 3) for interindividual variation and sensitive populations (*e.g.*, hypovitaminosis D individuals). Following a review of the available data on phosphorus and the effect of phosphorus (as phosphate) on the calcium-parathyroid hormone axis, the COT (2004a) concluded that there was insufficient data to indicate that the consumption of greater intakes of supplemental phosphorus affected parathyroid hormone levels and thus plasma calcium levels and bone health. Furthermore, a clinical trial with phosphated distarch phosphate demonstrated that the consumption of up to 60 g of phosphated distarch phosphate/day is well-tolerated in humans (Pieters *et al.*, 1971).

XII MICROBIOLOGICAL INFORMATION ON THE NOVEL FOOD

Based on the SCF guidelines, the following question must be addressed to ensure sufficient microbiological information on the novel food:

- "Is the presence of any microorganisms or their metabolites due to the novelty of the product/process?"

This question has been addressed collectively below.

Microbiological specifications are set for finished product RS4-fibre* and are provided in Section 1.8.1 above.

Details of the HACCP program in place during manufacture are discussed in Section II.6.

XIII TOXICOLOGICAL ASSESSMENT OF THE NOVEL FOOD

Based on the SCF guidelines, the following questions must be addressed to ensure sufficient toxicological information pertaining to the novel food:

- "Is there a traditional counterpart to the novel food that can be used as a baseline to facilitate the toxicological assessment?"
- "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?"
- "Is there information which suggests that the novel food might pose an allergenic risk to humans?"

These questions have been addressed collectively in Sections XIII.1 through XIII.4.

XIII.1 Toxicological Evaluation of Phosphated Distarch Phosphate

XIII.1.1 Introduction

The safety of RS4 fibre* can be assessed from a review of studies performed with phosphated distarch phosphate specifically (Corn Products Company, 1964; TNO and the Corn Refiners Association as summarised in table XIII.2.9 below), information pertaining to phosphorus (*i.e.*, various phosphorus-containing compounds) and other forms of starch (*e.g.*, monostarch and distarch phosphate) prepared using the same or similar treatments as used for phosphated distarch phosphate. Studies examining the effects of phosphorus were included to address any issues related to residual (bound) phosphate present in RS4-fibre*. Similarly, studies performed with other forms of modified starch (*e.g.*, monostarch phosphate and distarch phosphate) were included to address the effects of consuming resistant starches. Monostarch phosphate and distarch phosphate were included since phosphated distarch phosphate is prepared though a combination of treatments used to prepare monostarch phosphate and distarch phosphate, and since monostarch phosphate shares the same specification for residual phosphate (*i.e.*, maximum residual phosphate level of 0.4% for cereal starches). Although a number of reviews have been conducted on the safe intake of phosphorus (*e.g.*, JECFA, 1974d; IOM, 1997; EVM, 2003; COT, 2004b), a number of the traditional toxicology studies are unpublished and only the summaries are available from the reviews. The additional information pertaining to the safety of phosphorus and the safety of other modified starches provides supplementary safety information to address knowledge gaps related to the safety of phosphated distarch phosphate.

XIII.2 Phosphated Distarch Phosphate

XIII.2.1 Absorption, Distribution, Metabolism, and Excretion (ADME)

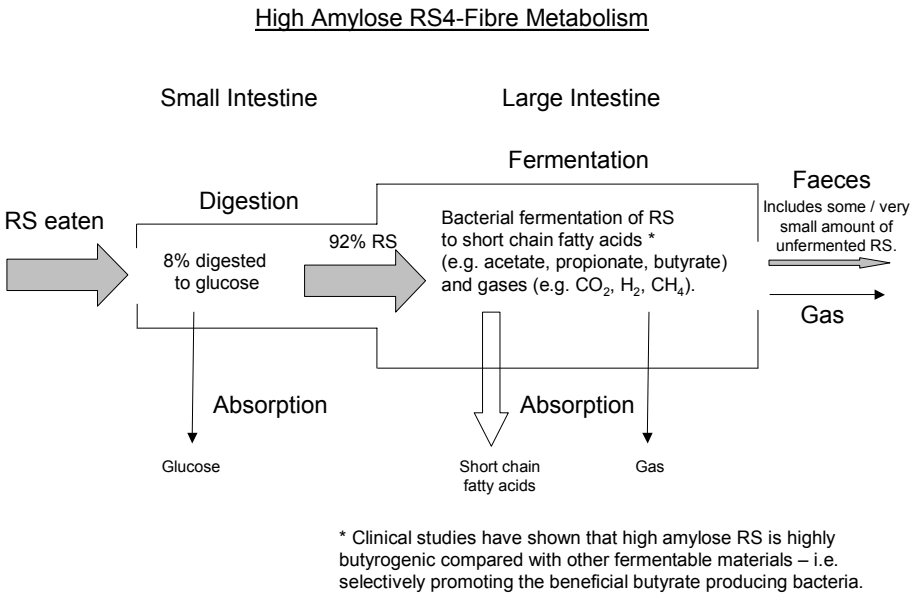
Compared with unmodified starch the *in vitro* digestibility of phosphated distarch phosphate and distarch phosphate (Kohn and Kay, 1963a starch (Leegwater, 1971) by pancreatic amylase or pancreatin and porcine, respectively, was reported to be somewhat reduced.

Alternatively, the *in vivo* digestibility and utilization of phosphated distarch phosphate and distarch phosphate (from maize) were reported to be similar to that of the unmodified starch (Kohn and Kay, 1963b). The digestibility and utilization of phosphated distarch phosphate was examined in 10 weaning male rats fed 5 g of the basal diet supplemented with 1, 2, or 4 g phosphated distarch phosphate or unmodified starch for 10 days with weight gain as the measured endpoint. Rats fed phosphated distarch phosphate were reported to have identical weight gains at all 3 levels of supplementation compared with rats fed the unmodified starch. Conversely, RS4-type fibres are more resistant to digestion (uncooked or cooked) compared to unmodified maize starch (cooked or uncooked) as illustrated in Figure XI.1-1. High amylose maize, as indicated by its name has a higher proportion of amylose *versus*

amylopectin thereby providing it with a greater resistance towards digestion. Brown *et al.* (2003) demonstrated that starch high in amylose are more able to retain their resistance towards digestion following cooking compared to starches high in amylopectin.

In a study of the metabolic behaviour of the phosphate radical in monostarch phosphate, rats were administered labelled starch phosphate, labelled orthophosphate or pyrophosphate (Laboratories of International Minerals & Chemicals Co., 1955). The distribution of p^{32} was examined with the percentage of activity retained in the liver, kidney, blood plasma, bone, as well as excreted in the urine and faeces determined. No significant differences were reported with respect to the distribution or excretion of ^{32}P in rats administered the 3 types of phosphate (*i.e.*, starch phosphate, orthophosphate, or pyrophosphate).

Figure XIII.2.1-1 Metabolism of Phosphated Distarch Phosphate



XIII.2.2 Acute Studies

No information is currently available on the acute oral toxicity of phosphated distarch phosphate. The acute oral toxicity of distarch phosphate, a modified starch that is prepared through esterification of starch with sodium trimetaphosphate or phosphorus oxychloride, was examined in mice, rats, guinea pigs, rabbits, and cats (10 animals/species) administered oral doses of 50% aqueous suspensions of distarch phosphate (Hodge, 1954, 1956). The reported LD_{50} values for distarch phosphate reported in 1954 were >24,000, >20,000, >8,800, and >7,000 mg/kg body weight in female mice, female rats, guinea pigs, rabbits, and cats,

respectively. Hodge (1956) reported the LD_{50} values for distarch phosphate as being 19,000, >35,000, >18,000, >10,000, and >9,000 mg/kg body weight in female mice, female rats; guinea pigs; rabbits; and cats, respectively. Hodge (1954, 1956) reported that no deaths occurred in the acute studies; however, only small numbers of animals were used. No histological abnormalities were reported in the livers and kidneys of guinea pigs, rabbits, and cats.

In a nutritional assay, the relative caloric value of phosphated distarch phosphate was compared with distarch phosphate, and white unmodified starch (reference supplement) (Kohn and Kay, 1963a). The rats were divided into 10 groups (10 rats/group) and fed a basal diet supplemented with 1.0, 2.4, or 4.0 g of phosphated distarch phosphate, distarch phosphate, or white unmodified starch (controls) over a 10-day assay period. An additional group was fed the 5.0 g/day basal diet without any supplemental starch. No abnormal behavioural reactions were reported during the pre-assay or assay periods, and weight gain was similar between the treatment groups (phosphated distarch phosphate and distarch phosphate) and the reference supplement groups (white unmodified starch).

XIII.2.3 Subchronic Studies

The potential effects of phosphated distarch phosphate and distarch phosphate were examined in weaning Pitman-Moore miniature pigs administered diets containing 5.4% unmodified starch (control), 5.6% phosphated distarch phosphate or distarch phosphate for 25 days (Anderson *et al.*, 1973a,b). The growth of the pigs fed 5.6% phosphated distarch phosphate or distarch phosphate was reported to be normal: blood (*i.e.*, haemoglobin), serum (*i.e.*, cholesterol, triglyceride, calcium, phosphorus, alkaline phosphatase, urea nitrogen, total protein, albumin, and globulin), relative organ weights, relative carcass (*i.e.*, water, fat, protein, ash, calcium, magnesium, phosphate, sodium) and liver (*i.e.*, water, fat, protein, ash) composition parameters were comparable between the treatment groups and control group.

Rats (10/sex/group) were administered 0, 25, or 50% modified starch (from maize) for 7 days in a low-residue diet (de Groot and Spanjers, 1970). The rats were administered an additional 4% cellulose for a further 3 days with no apparent adverse effects. The test animals (both sexes) were reported to have slightly reduced body weights in a dose-related manner; however, the actual change was small. The amount of faecal dry matter was slightly higher in animals administered the modified starch compared with controls, although no diarrhoea was reported at any level of modified starch administered. The caecal size of the rats administered modified starch was higher compared to controls, but no histological abnormalities of the enlarged caeca were reported.

In a follow-up study, de Groot and Spanjers (1970) administered 0, 25, or 50% modified starch (approximately 0.3% phosphate) to groups of 10 weanling Wistar-derived rats/sex/ treatment for 8 weeks. Body weight and faeces production were unaffected by the treatment

and no diarrhoea was reported at any test level. At the 50% test level faecal water content was reported to be higher, while at the 25% test level caecal weight was reported to be slightly increased in male rats.

Weanling rats (10/sex/treatment, strain not specified) were fed diets consisting of the basal mixture alone (control group) or the basal mixture plus 10% (approximately 30 g/kg body weight), rising by increments of 5% over the first 13 test days to 35% phosphated distarch phosphate from maize (approximately 70 g/kg body weight) for a total of 60 days (Kohn *et al.*, 1964a). Weight gain was reported to be consistently reduced in the female rats fed phosphated distarch phosphate compared with the control group, and the overall weight gain of the treated female group was significantly lower than the weight gained by the test group. A number of deaths were reported in the study (2 control and 4 treated) animals, which were attributed to natural causes and were considered unrelated to the test substance. The absolute kidney weights of both male and female rats, and the absolute liver weights of the male rats were significantly lower in the treatment group compared with the control groups, as were the kidney- and liver-body weight ratios; however, these results were deemed to be coincidental rather than related to the ingestion of the test starch. No gross or histopathological alterations were reported in association with the altered organ weights upon necropsy.

During a 90 day study, female Sprague-Dawley weanling rats were administered 0.20, 1.0, or 5.0% white unmodified starch base, control phosphate starch, or phosphated distarch phosphate (from maize) in their food, corresponding to doses of approximately 200, 1,000, or 5,000 mg/kg body weight (FDA, 1993) (Kohn *et al.*, 1964b). Each starch was fed at the 3 dose levels to 25 rats/sex/treatment. No adverse effects were reported on body weight gain, food consumption, food utilization, survival, behavioural patterns, haematological and urinalysis results, gross and microscopic pathological endpoints, or organ weights and ratios related to the test substance. Twenty rats died during the study period, 11 controls (unmodified starch base), 6 phosphate controls, and 3 phosphated distarch phosphate-treated rats; all deaths were attributed to respiratory disease.

In another 90-day rat study, 10 rats/sex were administered 0, 5, 15, or 45% of 2 types of distarch phosphate (0.085% esterified and 0.128% esterified phosphate) in their diet (Til *et al.*, 1970). No abnormalities were reported in the rats treated with the 2 types of distarch phosphate with respect to general appearance, behaviour, mortality, food consumption, haematology, serum chemistry, urinalysis, caecal weights, stool consistency (*i.e.*, no diarrhoea), gross and histopathology compared to the control group.

In a 90 day study, male and female beagle dogs (3/sex/group) were orally administered gelatine capsules containing white unmodified starch (control), 50, 250, or 1,250 mg phosphated distarch phosphate/kg body weight/day or equivalent doses of distarch phosphate (Cervenka and Kay, 1963). No adverse effects were reported in treated animals (*i.e.*,

phosphated distarch phosphate or distarch phosphate) with respect to body and organ weights, food consumption, mortality, heamatologic, urinalysis, liver function, and gross and microscopic pathologic findings. A death was recorded in the treatment group receiving 250 mg phosphated distarch phosphate; however, the death of the animal was attributed to canine distemper and therefore was unrelated to the test material.

XIII.2.4 Chronic Studies

de Knecht-Van Eekelen *et al.* (1971) fed a modified starch at levels of 0, 5, 10, and 30% (approximately 0, 5,000, 10,000, and 30,000 mg modified starch/kg body weight/day) to groups of 30 rats/sex for 104 weeks. The phosphated distarch phosphate was prepared through cross-linking of white unmodified maize starch with sodium trimetaphosphate (0.04% phosphorus) and esterified with sodium tripolyphosphate (total of 0.35% bound phosphorus). Growth rates, food efficiency, and relative organ weights were comparable with the controls except that males experienced significantly decreased spleen weights and females experienced significantly increased spleen and kidney weights at the highest level of modified starch (*i.e.*, 30%). The differences in organ weight were not associated with any gross pathological findings. No consistent changes were evident in haematology, serum chemistry, and urinalysis in relation to the test substance (*i.e.*, the modified starch). At all dietary levels, modified starch had no effect on caecal weights and there was no evidence of any carcinogenic effect or distinct compound-related changes upon histological examination. Among test and control animals, non-neoplastic lesions were randomly distributed except for a kidney abnormality consisting primarily of focal hyperplasia of the renal papillary and pelvic epithelium with calcified patches of underlying tissue. Test animals were reported to demonstrate a slightly higher incidence of nephrocalcinosis and hyperplasia of the pelvic epithelium compared with control animals, with 9 rats (5 male and 4 female rats) fed the 30% diet exhibiting this formation. de Knecht-Van Eekelen *et al.* (1971) did not consider the lesions to be of toxicological significance.

In an extensive review of pelvic nephrocalcinosis (PN) type mineral deposits in the renal pelvis of rats conducted by Roe (1977), the occurrence of PN in modified starch treated and untreated (control) animals were examined. Roe (1977) described 2 kinds of lesions that involve the deposition of minerals in the rat kidney. The first type of lesion has been described as small blue-grey mineral deposits identified in the corticomedullary region (*i.e.*, corticomedullary nephrocalcinosis) in the lumina of the tubules or in the connective tissue between the tubules without an inflammatory response or evidence of tissue reaction. The second type of lesion was described as large grey-blue mineral deposits in the epithelium in the renal pelvis, potentially associated with haemorrhage, thrombus, or hyperplasia and not frequently encountered in the corticomedullary region in untreated rats. Roe (1977) assigned the term PN to the second type of lesion for the purposes of simplicity in the report. No association was identified between the first type of lesion and the consumption of any

modified starch by the rats. Roe (1977) reported that there was some association between PN and various modified starches including phosphated distarch phosphate. The development of PN in the rats fed modified starch was complicated by the presence of parasitic worms (*Trichosomoides crassicauda*) in the rat colony and the use of a single strain of rats (Wistar) by CIVO. The parasite is harboured in the urinary tract and its presence pre-disposes rats to hyperplasia of the uroepithelium and calcus formation. In a subsequent publication, Roe (1979) concluded that PN, corticomedullary nephrocalcinosis, acute tubular nephropathy, and calcus formation are the result of mineral imbalances and are relatively common in untreated laboratory animals, particularly older animals.

XIII.2.5 Developmental and Reproductive Studies

In a 3-generation reproduction study, groups of 10 males and 20 female rats were fed 10% phosphated distarch phosphate [*i.e.*, maize starch modified with sodium trimetaphosphate (up to 0.01% phosphorus) and sodium tripolyphosphate (up to a total of 0.35% phosphorus)], acetylated distarch phosphate, acetylated diamylopectin phosphate, starch acetate, or hydroxypropyl distarch glycerol (Til *et al.*, 1971; de Groot *et al.*, 1974). The rats were mated at week 12 and week 20 post-weaning with the second litter of each additional generation used to produce the subsequent generation. Histopathological analysis was performed on the F_3b-generation, while the P, F_1b, and F_2b parents were used to count implantation sites. Appearance, behaviour, body weights, fertility, litter size, resorption quotient, pup weights and mortality were not adversely affected by modified starch consumption. The filled caecum weight of the F_1 parent males and the spleen weight of the F_3b females were increased; however, the caecal and organ weights of the other generations were not affected by modified starch consumption. No pathological changes attributable to modified starch consumption were evident upon gross and macroscopic examination. None of the modified starches were associated with reproductive effects.

XIII.2.6 Mutagenicity and Genotoxicity Studies

No information is currently available regarding the mutagenicity or genotoxicity of unmodified or modified starches. Chambers and Grand (1937, 1939) examined the effect of starch granules (type of starch not specified) injected into tumours and reported complete regression of the tumours. Conversely, numerous mutagenicity studies have been performed with various phosphorus-containing compounds (see Section XIII.2.7-1).

XIII.2.7 Human Studies

No adverse effects were reported in a group of 12 volunteers that consumed 60 g/day of phosphated distarch phosphate from maize (0.35% phosphorus) (approximately 1,000 mg/kg body weight/day) on 4 consecutive days (Pieters *et al.*, 1971). Frequency and the amount of faeces or faecal water and lactic acid content were reported to be unaffected.

XIII.2.8 Summary

Numerous studies have been performed with phosphated distarch phosphate, the majority of which have been performed with various animal species; however, one human tolerability study has been conducted. The type of study, the species/medium used, and the dose and duration of the studies conducted with phosphated distarch phosphate have been summarized in Table XIII.2.9-1.

Table XIII.2.9-1 Summary of Studies Performed with Phosphated Distarch Phosphate*

Type of Study	Species/medium	Dose & Duration	Reference
In vitro digestibility	Pancreatic amylase or pancreatin and porcine	NA	Kohn and Kay, 1963a (Corn Products 1964)
In vivo digestibility	Rat (male)	1, 2, or 4 g for 10 days	Kohn and Kay, 1963b (Corn Products 1964)
Subchronic toxicity	Pitman-Moore miniature pigs	5.6% for 25 days	Anderson *et al*., 1973a,b Corn Refiners Association
Subchronic toxicity	Rats	0, 25, or 50% for 7 days	de Groot and Spanjers, 1970 TNO
Subchronic toxicity	Rats	0, 25, or 50% for 8 weeks	de Groot and Spanjers, 1970 TNO
Subchronic toxicity	Rats	10% rising to 35% for a total of 60 days	Kohn *et al.*, 1964a (Corn Products 1964)
Subchronic toxicity	Rats	0.2, 1.0, or 5.0% for 90 days	Kohn *et al.*, 1964b (Corn Products 1964)
Subchronic toxicity	Rats	0, 5, 15, or 45% for 90 days	Til *et al*., 1970 (TNO)
Subchronic toxicity	Beagle dogs	0, 50, 250, or 1,250 mg/kg bw/day for 90 days	Cervenka and Kay, 1963b (Corn Products 1964)
Chronic toxicity	Rats	0, 5,000, 10,000, or 30,000 mg/kg bw/day for 104 weeks	de Knecht-Van Eekelen *et al.*, 1971 TNO – also published as De Groot *et al*. 1964
Developmental and Reproductive toxicity	Rats	10% during and post-gestation for 3 successive generations	de Groot *et al.*, 1974 TNO
Tolerability	Human volunteers	60 g/day for 4 consecutive days	Pieters *et al.*, 1971 TNO

NA = not applicable
* note all test articles used maize starch starting material and the same reaction process as RS4

XIII.3 Phosphorus-Containing Compounds

XIII.3.1 Absorption, Distribution, Metabolism, and Excretion (ADME)

In the small intestine absorption of phosphorus primarily occurs as inorganic phosphate (IOM, 1997). The greatest absorption of phosphorus has been reported in the jejunum with absorption decreasing along the length of the small intestine (EVM, 2003). The net absorption of phosphorus in adults and infants/children from a mixed diet has been reported to range from 55 to 70% and 65 to 90%, respectively (IOM, 1997). Studies conducted by Stanbury (1971) and Lemann (1996) examining the relationship of adult faecal phosphorus excretion and dietary intake of phosphorus demonstrated that there is no apparent adaptive mechanism that improves phosphorus absorption at low intakes (IOM, 1997). This is distinctly different from calcium, which is subject to active absorption *via* a Ca^{2+}-Na^{+}ATPase-pump to improve calcium absorption during periods of low calcium intake (IOM, 1997). Although studies have demonstrated that a portion of phosphorus absorption occurs *via* saturable, active transport facilitated by 1,25-dihydroxyvitamin D, the majority of phosphorus is absorbed under passive, concentration-dependent processes.

The level of phosphorus and calcium in the blood is primarily regulated by PTH and is affected by the dietary intake of calcium and phosphorus (EVM, 2003). The majority of phosphorus in the body (approximately 80%) is sequestered in the skeleton with the remainder present in soft tissues and extracellular fluid. In the blood phosphorus is predominantly a constituent of the phospholipids (approximately 70% of phosphorus present in the circulation) while the remainder is in the form of inorganic phosphate (*i.e.*, approximately 85% free and 15% protein-bound).

Phosphorus is primarily excreted in the urine with small amounts lost in cells shed from the skin and intestinal mucosa (IOM, 1997; EVM, 2003). The kidney acts as the main site of regulation for the retention/excretion of plasma phosphorus with inorganic serum phosphate filtered at the glomerulus and reabsorbed at the proximal tubule (IOM, 1997). The reabsorption of phosphate in the proximal tubule is limited and cannot exceed a certain number of mmole per unit time known as the tubular maximum for phosphate or TmP. The transport capacity of the proximal tubule varies inversely with PTH concentration, which acts to adjust the renal clearance of inorganic phosphate. High plasma concentrations of inorganic phosphate result in filtered loads of plasma with inorganic phosphate concentrations above the TmP whereby urinary phosphorus excretion is a linear function of plasma phosphate. Conversely, low plasma concentrations of inorganic phosphate result in filtered loads of plasma with inorganic phosphate concentrations below the TmP and most or the entire filtered load is reabsorbed in an attempt to maintain plasma concentrations of inorganic phosphate.

XIII.3.2 Acute Studies

Numerous acute toxicity studies have been conducted with phosphorus-containing compounds such as phosphoric acid, phosphates, orthorphophates, tetrasodium diphosphates, triphosphates, polyphosphates, and calcium phosphate. The approximate LD_{50} values for the predominantly unpublished studies have been summarized in Table XIII.3.2-1. The results of these acute toxicity studies indicate that phosphorus consumed as inorganic phosphates is not particularly toxic.

Table XIII.3.2-1 Oral Acute Toxicity LD_{50} Values for Phosphorus-Containing Compounds

Compound	Species	LD_{50} (mg/kg bw)	Approximate phosphorus equivalence (mg/kg bw)	Reference
Phosphoric acid, Phosphates, and Orthophosphates				
Monosodium phosphate (NaH_2PO_4)	Guinea pig	2,000	516.34	Eichler, 1950
Monosodium phosphate (NaH_2PO_4)	Mouse	3,700	955.22	FDRLI, 1975a
	Rat	4,100	1,058.49	
Monopotassium phosphate (KH_2PO_4)	Mouse	3,200	728.35	FDRLI, 1975b
	Rat	2,820	641.86	
Sodium acid pyrophosphate ($Na_2H_2P_2O_7$)	Mouse	3,350	467.53	FDRLI, 1975a
	Rat	1,690	235.86	
	Hamster	1,660	231.67	
Tetrasodium Diphosphate				
$Na_4P_2O_7$	Mouse	1,300	151.43	FDRLI, 1975c
	Rat	1,380	160.75	
Triphosphates and Polyphosphates				
Sodium triphosphate ($Na_2H_3P_3O_{10}$)	Mouse	2,380	244.17	FDRLI, 1975b
	Rat	1,700	174.41	
	Rabbit	2,500	256.48	
1/3 Kurrol's salt and 2/3 tetra- and disodium Diphosphate (H_2O soluble, neutral)	Rat	4,000	NC[a]	van Each *et al.*, 1957
	Rat	18[b]	NC[a]	
Sodium hexametaphosphate ($Na_6H_6P_6O_{18}$)	Mouse	3,700	187.33	FDRLI, 1975d
	Rat	2,400	121.51	
Calcium Phosphate				
Monocalcium phosphate ($CaHPO_4$)	Mouse	4,600	1047.22	FDRLI, 1975c
	Rat	2,170	494.01	

Adapted from: JECFA (1982b)
[a] NC = Not calculated since insufficient information provided with regard to the composition of the compound
[b] Rats dosed intravenously

XIII.3.3 Subchronic Studies

In addition to the subchronic and chronic studies performed with phosphated distarch phosphate and distarch phosphate, a number of subchronic toxicity studies have been conducted with various phosphorus-containing compounds. The occurrence of adverse effects was reported with the administration of phosphorus-containing compounds such as decreased growth, the occurrence of PN, and kidney damage. The design and results of the subchronic studies for the various phosphorus-containing compounds have been summarized in Table XIII.3.3-1.

Table XIII.3.3-1 Subchronic Toxicity Studies for Various Phosphorus-Containing Compounds

Compound	Species	Design	Effect	Reference
Phosphoric acid, Phosphates, and Orthophosphates				
Dicalcium phosphate or ammonium polyphosphate	5 sheep/ treatment	- basal diet containing 11 g of dicalcium phosphate or 6.25 g of ammonium polyphosphate (urea added as a nitrogen source) - diets fed for 1 week at a time	- addition of either phosphate source increased appetite, weight gain, and apparent nitrogen retention	Fishwick, 1974
Dibasic potassium phosphate (K_2HPO_4)	12 rats/ group	- 3 groups: control (0.56% Ca, 0.42% P), normal orthophosphate (0.47% Ca, 0.43% P), high orthophosphate (0.50% Ca, 1.30% P)	- No adverse effects at 50, 60, and 150 days of observation	Dymsza *et al.*, 1959
Disodium Phosphate and Tetrasodium Phosphate				
$Na_4P_2O_7$ or monophosphate	20 rats/ group (male and female)	0 (control), 1%, 2.5%, or 5% $Na_4P_2O_7$, or 5% monophosphate added to rat diets for 16 weeks	- 2.5% $Na_4P_2O_7$ level and above kidney weight was increased and kidney function reduced - greater incidence of kidney damage in 1% group compared with controls - more severe kidney damage at higher concentration of $Na_4P_2O_7$ - stomach hypertrophy and haemorrhages at higher $Na_4P_2O_7$ concentrations (not in 5% monophosphate groups)	Datta *et al.*, 1962
$Na_4P_2O_7$ or sodium monophosphate	34 to 36 young rats	1.8%, 3%, or 5% $Na_4P_2O_7$ or sodium monophosphate added to a modified Sherman diet for 6 months	- growth significantly decreased and appearance of nephrocalcinosis with 3% and 5% diphosphate diets - similar degree of kidney damage with either phosphate source	Hahn *et al.*, 1958; Hahn and Seifen, 1959

Table XIII.3.3-1 Subchronic Toxicity Studies for Various Phosphorus-Containing Compounds

Compound	Species	Design	Effect	Reference
Triphosphates and Polyphosphates				
Sodium hexametaphosphate or sodium tripolyphosphate	5 male rats/group	- 0.2%, 2%, or 10% sodium hexametaphosphate or sodium tripolyphosphate - controls given 10% sodium chloride or 5% disodium phosphate - 1 month study duration	- growth retardation, increased kidney weights, and tubular necrosis reported with 10% of either of the polyphosphates or NaCl - no deaths with 10% of either polyphosphate - 2% polyphosphate resulted in inflammatory changes in kidneys, but growth unaffected - no adverse effects with 0.2% polyphosphate	Hodge, 1956
Disodium ($Na_2H_2P_2O_4$) and Tetrasodium diphosphate ($Na_4P_2O_7$)	10 rats/sex/ group	0.5%, 1%, 2.5%, or 5% Kurrol's salt plus $Na_2H_2P_2O_4$ or $Na_4P_2O_7$ added to Sherman diet for 12 weeks	- normal growth with 0.5%, 1%, and 2.5% of either polyphosphate - growth retardation with 5% of either polyphosphate - kidney weights slightly increased with 1% polyphosphate, and further increased with 2.5% and 5% - histopathological analysis showed nephrocalcinosis and extensive tubular tissue damage occurred in kidneys of 5% animals, with lesser damage present in 2.5% group, and no damage with 0.5% dose	van Each *et al.*, 1957; van Genderen, 1958
Ammonium polyphosphate	Pigs	Replaced 50% or 100% defluorinated rock phosphate in diet with ammonium polyphosphate for 13 weeks	No influence of ammonium polyphosphate on daily feed intake, rate of gain, or feed: gain ratio compared with controls	Clawson and Armstrong, 1981
Sodium hexametaphosphate	12 male rats/group	0.9% and 3.5% sodium hexametaphosphate (0.46% and 1.20% phosphorus, respectively) or potassium monophosphate (control) for 150 days	- growth, food, and protein efficiency were poorest with 3.5% added hexametaphosphate - 3.5% hexametaphosphate group kidneys were heavier compared to control rats - no histopathology abnormalities in the kidneys from any of the groups	Dymsza *et al.*, 1959

Table XIII.3.3-1 Subchronic Toxicity Studies for Various Phosphorus-Containing Compounds

Compound	Species	Design	Effect	Reference
Sodium tripolyphosphate or Graham's salt	36 rats/sex/ group	3% or 5% sodium tripolyphosphate or 1.8%, 3%, or 5% of Graham's salt added to modified Sherman diet for 24 weeks (control group supplemented with orthophosphate)	- temporary growth inhibition and growth retardation reported with 3% and 5% of either preparation, respectively - normal growth in male rats with 1.8% Graham's salt - nephrocalcinosis reported in 3% and 5% groups - degree of damage less with Graham's salt and equivalent with tripolyphosphate compared with control	Hahn *et al.*, 1956; Hahn *et al.*, 1958; Hahn and Seifen, 1959
Sodium tripolyphosphate ($Na_5P_3O_{10}$), sodium hexametaphosphate	4 dogs	- 0.1 g/kg bw/day $Na_5P_3O_{10}$ and sodium hexametaphosphate were fed to 1 dog each for 1 month - 2 other dogs (1/compound) were fed $Na_5P_3O_{10}$ or sodium hexametaphosphate at a starting dose of 1.0 g/kg bw/d and increased to 4.0 g/kg bw/d at the end of the 5-month study period	- sodium hexametaphosphate-treated dog began to lose weight when dose reached 2.5 g/kg bw/d - $Na_5P_3O_{10}$-treated dog only lost weight when dose reached 4.0 g/kg bw/d - urinalysis, haematology, and organ weights were normal in both groups, except for the $Na_5P_3O_{10}$-treated dog, which had an enlarged heart (hypertrophy of the left ventricle) - tubular damage noted in the kidneys of both dogs at higher doses - no tissue changes attributed to 0.1 g/kg bw/d treatment	Hodge, 1956

Adapted from: JECFA (1982b)

XIII.3.4 Chronic Studies

Chronic studies performed with various phosphorus-containing compounds have been conducted with adverse effects reported with the administration of phosphorus-containing compounds such as decreased growth, the occurrence of PN, increased rate of bone turnover, increased PTH, and kidney damage. The design and results of the subchronic studies for the various phosphorus-containing compounds have been summarized in Table XII.3.4-1.

Table XIII.3.4-1 Chronic Toxicity Studies for Various Phosphorus-Containing Compounds

Compound	Species	Design	Effect	Reference
Phosphoric acid, Phosphates, and Orthophosphates				
Phosphoric acid	rats (3 successive generations)	Administered diets containing 0.4% or 0.75% for 90 weeks	Growth and reproduction were unaffected No pathological or blood chemistry differences between the treated and control groups Dental attrition more marked in treated compared to control rats	Lang, 1959
Phosphoric acid	rats (> 700)	Rats were fed diets containing 0.75% phosphoric acid and followed through 3 generations	No adverse effects upon examination or in the blood, tissues, mineral balance, nitrogen retention, or acidic conditions of the digestive tract were reported	Ellinger, 1972
Disodium Phosphate and Tetrasodium Diphosphate				
$Na_2H_2P_2O_7$ (2/3) and Kurrol's salt (1/3)	10 rats/group/ sex	Provided a Sherman diet with 0.5%, 1%, or 5% $Na_2H_2P_2O_7$ and Kurrol's salt (through 3 generations)	- no effects on growth, fertility, or average lifespan - nephrocalcinosis occurred at 1% and 5% levels - frequency of tumours similar between controls and treated	van Each *et al.*, 1957
Triphosphates and Polyphosphates				
1/3 Kurrol's salt and 2/3 diphosphate	30 male and 10 female rats	Mixture of 1/3 Kurrol's salt and 2/3 diphosphate in concentrations of 0.5%, 1%, 2.5%, or 5% added to Sherman diet (entire lifespan)	- 2 successive generations produced 1st and 2nd generation growth significantly inhibited at 5% dietary level - fertility was lower at the 5% dietary level while 0.5%, 1%, and 2.5% dietary level had no effect - decreased number of erythrocytes in the second generation 2.5% group - no kidney damage attributed to polyphosphate treatment in the 0.5% group - kidney calcification occurred in higher dose groups, with the degree of damage increasing with dose	van Each *et al.*, 1957

Table XIII.3.4-1 Chronic Toxicity Studies for Various Phosphorus-Containing Compounds

Compound	Species	Design	Effect	Reference
Sodium tripolyphosphate	50 weanling rats/sex/group	0.05%, 0.5%, or 5% sodium tripolyphosphate added to the rats diet for 2 years	- growth significantly reduced in males, but only slightly in females at the 5% polyphosphate level - survival reduced in the 5% group compared with the other groups - increased kidney weight in the 5% group - reproduction and fertility normal in the 0.5% polyphosphate and control groups - no pathological changes attributed to treatment in 0.05% or 0.05%	Hodge, 1960a
Sodium hexametaphosphate	50 weanling rats/sex/group	0.05%, 0.5%, or 5% sodium hexametaphosphate added to the rats diet for 2 years	- growth retardation only apparent in 5% group - mortality high in all groups (*i.e.*, unrelated to dose) - rats in 5% group had higher kidney weights and calcification was present - kidneys and reproduction unaffected in the 0.5% group	Hodge, 1960b
Calcium to Phosphorus ratios				
Phosphorus	Pigs	Adult pigs fed basal diets containing 0.65% calcium with phosphorus added at 1, 2, or 3 times the amount of calcium for 6 months	- decreased weight gain, slight hypocalcaemia, significant hyperphosphatemia, increased PTH, and increased rate of bone turnover with diets containing calcium to phosphorus ratios of 1:2 and 1:3 - total kidney calcium and phosphorus increased with 1:3 diet	De Luca *et al.*, 1976
Phosphorus	monkeys (cinnamon ringtail)	Provided monkeys high phosphorus-containing diets (up to 1:4 calcium to phosphorus)	- minor (microscopic) bone changes not apparent upon gross examination	Anderson *et al.*, 1977

XIII.3.5 Developmental and Reproductive Studies

In addition to the developmental and reproductive studies performed with phosphated distarch phosphate, a number of developmental and reproductive studies have conducted with various phosphorus-containing compounds. No adverse effects on the dams or offspring

were reported with the administration of the various phosphorus-containing compounds. The design and results of the studies have been summarized in Table XIII.3.5-1.

Table XIII.3.5-1 Reported Results of Reproductive and Teratogenicity Studies of Phosphorus-Containing Compounds

Compound	Species	Dose (mg/kg bw)	Results	Reference
Monosodium phosphate	Mouse	370	- no maternal toxicity or teratogenic effects[1]	FDRLI, 1975a
	Rat	410		
Monopotassium phosphate	Mouse	320	- no maternal toxicity or teratogenic effects	FDRLI, 1975b
	Rat	282		
Sodium acid pyrophosphate	Mouse	335	- no maternal toxicity or teratogenic effects	FDRLI, 1973a
	Rat	169		
	Hamster	166		
	Rabbit	128		
Tetrasodium Diphosphate	Mouse	130	- no maternal toxicity or teratogenic effects	FDRLI, 1975c
	Rat	138		
Sodium hexametaphosphate	Mouse	370	- no maternal toxicity or teratogenic effects	FDRLI, 1975d
	Rat	240		
Sodium tripolyphosphate	Mouse	238	- no maternal toxicity or teratogenic effects	FDRLI, 1973b
	Rat	170		
Monocalcium phosphate	Mouse	465	- no maternal toxicity or teratogenic effects	FDRLI, 1973c
	Rat	410		

[1] Maternal toxicity and teratogenic effects are absent up to the dose tested.

XIII.3.6 Mutagenicity and Genotoxicity Studies

Numerous unpublished genotoxicity and mutagenicity studies have been performed with inorganic salts of phosphorus *in vitro*, and all tests have demonstrated that the assorted forms of inorganic phosphorus assayed were not mutagenic (Newell *et al.*, 1974; Litton Bionetics Inc., 1975a,b,c; FDRLI, 1975d). Table XIII.3.6-1 contains a summary of the *in vitro* and *in vivo* genotoxic/mutagenic studies performed on inorganic salts of phosphorus.

Table XIII.3.6-1 Reported Results of *In Vitro* and *In Vivo* Genotoxic/Mutagenic Studies of Phosphorus-Containing Compounds

Compound	Test Object	End point/Assay	Result	Reference
Monocalcium phosphate	*Salmonella typhimurium* (TA1535, TA1537, TA1538)	Reverse mutation	Negative (+/-)[1]	Litton Bionetics Inc., 1975a,b,c
	Saccharomyces cerevisiae (D4)	NR[2]	Negative (+/-)	
Sodium acid pyrophosphate	Mouse	Host-mediated assay using *S. typhimurium* TA1530 or mitotic recombination frequency in *S. cerevisiae*[3]	Negative	Newell *et al.*, 1974
	S. typhimurium TA1535, TA1536, TA1537, TA1538	Reverse mutation	Negative (+/-)	
	Rat	Dominant lethal test	Negative	
	Rat	Translocation test	Negative	
Tetrasodium pyrophosphate	*S. cerevisiae* D4	NR	Negative (+/-)	FDRLI, 1975d
	S. typhimurium TA1535, TA1537, TA1538	Reverse mutation	Negative (+/-)	
Sodium tripolyphosphate	Mouse	Host-mediated assay using *S. typhimurium* TA1530 or mitotic recombination frequency in *S. cerevisiae* D3[3]	Negative	Litton Bionetics Inc., 1974
	S. typhimurium TA1530 and G46, and *S. cerevisiae* D3	Reverse mutation	Negative (+/-)	
	rat bone marrow cells[2] and human lung cells (*in vitro*)	Cytogenic study	Negative	
	Rat	Dominant lethal study	Negative	
Sodium hexametaphosphate	*S. typhimurium* TA1535, TA1536, TA1537	Reverse mutation	Negative (+/-)	Litton Bionetics Inc., 1975c

[1] With and without metabolic activation
[2] NR = not reported
[3] *In vivo*

XIII.3.7 Human Studies

The use of oral phosphate in the treatment of osteoporosis was examined in 79 postmenopausal women randomized in a double-blind study and provided with effervescent

tablets containing ammonium phosphate, potassium phosphate, or glycerol phosphate (Brixen *et al.*, 1992). Phosphorus was provided to 19, 19, and 21 subjects in amounts of 750 (low), 1,500 (mid), and 2,250 (high) mg phosphorus/day, respectively, for 7 days with a 4-month follow-up. A significant rise in serum PTH was reported in the groups provided with 1,500 and 2,250 mg of phosphorus/day, but not in the lowest dose. The ratio of urinary phosphate to creatinine was reported to increase in a dose-related fashion; however, no significant alteration was reported in the serum level of phosphate or calcium. The level of osteoclacin was increased in the 1,500 mg of phosphorus/day group, but not in the low- or high-dose groups. The increased osteoclacin activity in the mid-dose group was likely a chance finding and does not provide sufficient biochemical evidence for increased bone turnover resulting from phosphorus supplementation. Gastrointestinal side effects (*e.g.*, nausea, vomiting, loose stools, and diarrhoea) was reported by 2, 3, and 7 subjects in the low-, mid-, and high-dose groups, respectively. In the high-dose group, one other subject reported dizziness on a single occasion, while another patient discontinued treatment due to nausea. Aside from these isolated incidents, the side effects were mild and the subjects were able to complete the treatment course.

Lauersen (1953) examined the effect of phosphoric acid consumption in fruit juice on 15 students over 10 days, and in 2 additional males over 14 days. The 15 students were provided with 2,000 to 4,000 mg of phosphoric acid consumed in fruit juice every day for 10 days. The 2 male individuals were provided with 3,900 mg of phosphoric acid/day. The composition of the volunteer's urine was examined to determine if increased phosphoric acid consumption resulted in any metabolic disturbances. No alterations were reported in the urine of the volunteers. Lauersen (1953) also reported that the consumption of 6,000 mg of NaH_2PO_4 $2H_2O$/day was well-tolerated for 15 days without the occurrence of any adverse effects. Similarly, no adverse effects were reported with the extended daily intake of 5,000 to 7,000 mg of NaH_2PO_4 (approximately 1,500 mg of phosphorus) (Lang, 1959).

The effect of phosphorus supplementation on calcium homeostasis and bone turnover was examined in 2 studies with 10 and 12 healthy men participating in the 1st and 2nd study, respectively (Whybro *et al.*, 1998). The 1st study was a randomized, controlled crossover study with 1,000 mg phosphorus (sodium acid phosphate tablets) provided to the subjects for 1 week in addition to a standard diet containing 800 mg/day of calcium and phosphorus. The subjects in the 2nd study were provided with escalating doses of phosphorus, rising from 0 to 1,000, 1,500, and 2,000 mg/day (sodium acid phosphate tablet) in addition to a standard diet containing 1,000 mg/day of both calcium and phosphorus. The subjects ingested each dose level for 1 week. PTH levels in the serum were elevated in the first study, but unaffected at any dose (up to 2,000 mg) in the second study. Also, serum phosphate levels were unaffected by phosphorus supplementation in the second study. The only adverse effect reported in either study was the occurrence of diarrhoea in 1 subject in the 2nd study. Whybro *et al.*

(1998) concluded that bone turnover in young men is unaffected by phosphate supplementation at the doses tested.

The physiological response of adults to phosphorus as a food additive was examined in 8 healthy adult subjects provided with 2 g of total phosphorus for 4 weeks following a 4 week control period where the subjects consumed foods free of phosphate additives (Bell *et al.*, 1977). Serum and urinary calcium were reported to be decreased during the phosphorus supplementation period, while hydroxyproline excretion was increased and cyclic AMP was elevated in 6 out of 8 subjects. Soft stools and mild diarrhoea was reported during the 1st week of study with some subjects experiencing abdominal discomfort. The majority of subjects were symptom-free after the 1st week; however, mild gastrointestinal disturbances were reported by 2 subjects throughout the entire study period.

Similarly, the physiological effects of a phosphorus-rich diet was examined in 10 healthy young women during a 6-week supplementation period with 3,008 mg of phosphorus/day and 1,995 mg of calcium/day (Grimm *et al.*, 2001). During an initial 4-week baseline period the subjects were provided with a standard commercial diet containing 1,700 mg of phosphorus/day and 1,500 mg of calcium/day. The subjects participated in the supplementation period for a duration of 4 weeks and were provided with 3,008 mg of phosphorus/day and 1,995 mg of calcium/day. After the supplementation period the subjects were provided with the standard commercial diet for an additional 4 weeks. During the supplementation period non-significant increases were reported in serum PTH levels, serum 1,25-$(OH)_2D_3$, and urinary deoxypyridinoline, as well as a non-significant decrease in urinary microalbumin and a significant decrease in serum osteoclacin levels. Grimm *et al.* (2001) concluded that healthy women with normal calcium and excessive phosphorus intakes does not affect bone turnover as demonstrated by the non-significant changes in bone-related hormones and other biomarkers of bone turnover. Intestinal distress, soft stools or mild diarrhoea were reported by all of the study subjects during the 6-week supplementation period.

A slight decrease in the urinary excretion of calcium was reported in 4 men consuming a basal diet with 450 mg of calcium and 1,400 mg of phosphorus, and supplemented with 750 mg of phosphorus (as phosphoric acid) during a 1-week study period (Malm, 1953). During 12 weeks of supplementation a further decrease in urinary calcium excretion was reported.

Multiple myeloma patients were provided with phosphate supplements that contained 1,000 to 2,000 mg of phosphorus as an adjunct in multiple myeloma therapy (Goldsmith *et al.*, 1968). The 14 patients took daily oral doses of the phosphate supplements for up to 15 months or intravenous doses for an unspecified amount of time. Goldsmith *et al.* (1968) reported that bone pain and urinary calcium excretion were reduced and that there was no

evidence of extra-skeletal calcification during follow-up periods. Pedal and pretibial oedema was reported in one patient; however, the condition subsided with the discontinuation of phosphate salt supplement intake. Dyspepsia was reported by 1 other patient following the ingestion of the phosphate supplements, as well as after other medications including the placebo.

The effect of oral sodium phosphate ingestion on the formation of renal calculi and on idiopathic hypercalcuria was examined in 10 male and female patients afflicted with idiopathic hypercalcuria (Bernstein and Newton, 1966). The subjects were provided with 3.3 to 9.9 g of oral sodium phosphate for 4 months to 5 years. Urinary calcium excretion was reported to decrease 48.8% and the formation of stones was decreased during sodium phosphate therapy. Some subjects reported mild to moderate diarrhoea.

Goldsmith *et al.* (1976) also examined the effect of oral phosphate therapy on 7 postmenopausal women with osteoporosis. The subjects were provided with a buffer pair of potassium phosphates, which provided a daily dose of approximately 1 g of phosphorus, taken in divided doses with meals for more than 12 months. The subjects were reported to consume approximately 54 to 86 mmol and 20 to 37 mmol of phosphorus and calcium, respectively. Fasting serum concentrations of calcium and PTH levels were unaffected by phosphorus supplementation. Alternately, serum phosphate levels, urinary calcium, and tubular resorption of phosphorus were decreased. The number of bone resorption surfaces was reported to increase in all patients while the number of bone-forming surfaces decreased. Goldsmith *et al.* (1976) reported that bone-resorbing surfaces were highly correlated with phosphorus intakes.

XIII.4 Additional Data to Support Safety

The Scientific Committee for Food (SCF) reviewed the use of modified starches in 1976 and 1981 (SCF, 1976; 1981) and concluded that the use of modified starches in food was acceptable. Following a review of all of the available literature, including a thorough review of the formation of PN in rats, the SCF stated that the consumption of phosphated distarch phosphate at the current level of technological use was safe and did not necessitate the establishment of an ADI. Furthermore, the occurrence of PN and other forms of mineral deposition were not of toxicological relevance for humans with regard to phosphated distarch intake since the rat was particularly sensitive to mineral imbalances and the formation of PN was common in untreated laboratory controls. Although these conclusions were in the context of food additive use and clearly the proposed nutritional uses in this dossier exceed technological levels as thickeners in most cases, the second SCF report set a maximum limit of 5% for modified starches in foods for infants and young children. Currently, under

Directive 95/2/EC phosphated distarch phosphate is permitted in weaning foods[1] for infants and young children to a maximum amount of 50g/kg[2]. Whilst we clearly note here that we do not intend the current application to include infants within its scope, it is worth noting that the consumption of, for example, 200 g of baby jar food could potentially legally contain 10 g of E1413 (*i.e.,* RS4 fibre). Assuming a 10 kg body weight this would have a daily intake of up to 1 g/kg body weight. This would be equivalent to 60 g per day for and adult and would be in line with the above clinical study dosing levels (Pieters *et al.* 1971).

The safe upper level of intake of phosphorus, though the diet and consumption of dietary supplements has been examined by various international bodies including the Joint WHO/FAO Expert Committee on Food Additives (JECFA, 1974d), the Institute of Medicine (IOM, 1997), and the Expert Group on Vitamin and Minerals (EVM, 2003).

JECFA (1974d) concluded that the best estimate at which phosphorus intake would result in nephrocalcinosis in humans would be an approximate intake of 6,600 mg phosphorus/day. This value is based on the extrapolation from the lowest level of phosphorus in the rat diet (*i.e.*, 1% phosphorus) at which nephrocalcinosis resulted. JECFA (1974d) noted that rats are highly susceptible to calcification and hydronephrosis upon exposure to acids forming calcium chelates or complexes and that the dose levels at which nephrocalcinosis occurred were not consistent. The best estimate for an acceptable daily intake of total dietary phosphorus (*i.e.*, natural and food additive sources) was in the range of 0 to 70 mg/kg body weight.

The IOM (1997) reviewed the safety and metabolism of phosphorus in order to establish the safe upper level (UL) of intake for phosphorus in different age groups. Potential effects of excessive intakes of phosphorus were identified including adjustments in calcium-regulating hormones, metatastic calcification, skeletal porosity, and interference with calcium absorption. The majority of these adverse effects were observed in animal studies, which have a higher phosphorus density compared to human diets. According to the IOM (1997) there are no reports of adverse effects resulting from high dietary phosphorus intakes in humans. Instances of hyperphosphatemia and resultant adverse effects have resulted due to non-dietary reasons (*e.g.*, end-stage renal disease, vitamin D intoxication, *etc*.). The IOM (1997) calculated an UL of 4.0 g of phosphorus/day for adults aged 19 to 70 and adolescents aged 9 through 18 years, as well as 3.0 g of phosphorus/day for children aged 1 to 8 years and adults older than 70 years of age. Individuals with a higher level of energy expenditure (*i.e.*, >6,000 kcal/day) may have phosphorus intakes above the UL; however, no harm is expected to result from the higher intake. Males aged 14 to 18 years were identified as having the

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[1] Weaning foods**;** foodstuffs used as part of a diversified diet and not constituting to the sole source of nourishment.
[2] Directive 95/2/EC, Annex VI, Part 3

highest reported intake of 2.5 g phosphorus/day, which is well below the UL for that age group.

In addition to the evaluations performed by JECFA (1974d) and IOM (1997), the EVM (2003) examined the safety of phosphorus and performed an exposure assessment in order to establish a guidance level for phosphorus. The EVM calculated a maximum estimated daily intake of phosphorous of approximately 3,200 mg/day based on a mean and 97.5th percentile intake of phosphorous from food as 1,260 and 2,110 mg/day, respectively, and 1,100 mg/day from supplements. The EVM (2003) noted that there was insufficient data on which to base a Safe Upper Level for inorganic phosphates; however, the Committee established a guidance level of 250 mg/day (approximately 4.2 mg/kg body weight for a 60 kg adult), which is not expected to result in any adverse effects. The guidance value was based on a no observed adverse effect level (NOAEL) of 750 mg of phosphorus/day (Brixen *et al.*, 1992), and the application of an uncertainty factor of 3 to account for inter-individual variation (*e.g.*, people with hypovitaminosis D which are vulnerable to hyperparathyroidism). A few studies reviewed by the EVM (2003) reported subjects experiencing diarrhoea and mild gastrointestinal symptoms, which were reversible upon discontinuation of the doses of 750 to 2,250 mg/day.

In addition, the studies reviewed by the EVM (2003) and COT (2004b) utilized doses of supplemental phosphorus in pill or drink form thereby resulting a bolus dose of phosphorus. The ingestion of supplemental phosphorus was well-tolerated; however, mild effects including diarrhoea, soft stools, abdominal discomfort, and gastrointestinal discomfort. Grimm *et al.* (2001) suggested that the observed effects in the subjects resulted from the osmotic effect of the added polyphosphates in the intestinal lumen, which are absorbed in the range of 20 to 80% depending upon their chemical form. In contrast, exposure to phosphorus from phosphated distarch phosphate will be in meal form as part of a food matrix rather than a bolus dose in pill or drink form in addition to a meal. The occurrence of gastrointestinal effects should be lessened in such a case. Furthermore, the amount of phosphorus ingested in one sitting based on the estimated daily intake of phosphated distarch phosphate from individual proposed food-uses by different population groups within the United Kingdom will be well below the 750 mg/day of phosphorus reported to elicit gastrointestinal discomfort in human tolerance studies, and the 250 mg of phosphorus/day assigned as the guidance value for safe consumption of phosphorus. The lowest observed adverse effect level (LOAEL) was reported by Brixen *et al.* (1992) where subjects ingested either a placebo, 750 mg phosphorous (as ammonium phosphate) in a capsule in the morning and a placebo at lunch or at dinner, or 750 mg phosphorous 3 times daily (*i.e.*, at meal times). The estimated mean and 97.5th daily all-user intake of phosphorous from RS4-fibre* in male teenagers and male adults is approximately 129.5 and 288.7 mg/day, respectively. Assuming that the consumption of RS4-fibre* is evenly divided between meals and that an individual consumes 3 meals per day, the highest exposure would occur in male adults at the 97.5th percentile with

approximately 96.2 mg/meal of phosphorus with the consumption of 24.1 g/meal of RS4-fibre*. The 97.5th percentile intake of RS4-fibre* by individuals in the male adults group is estimated at 72.2 g/person/day. The consumption of 60 g/person/day of phosphated distarch phosphate was reported to be well-tolerated in 12 volunteers for 4 consecutive days (Pieters *et al.*, 1971). The other population groups are not expected to consume greater than 60 g/person/ day of phosphated distarch phosphate at the 97.5th percentile except for teenage males who are expected to consume slight less RS4-fibre* than the adult males.

The Committee on Toxicity (COT) examined data on phosphate, PTH secretion, calcium balance, and bone health following a disagreement between industry and the Food Standards Agency (FSA) regarding labelling (COT, 2004b). The FSA proposed adding statements to the label regarding the potential effect of phosphate on the gastrointestinal tract and that long-term use of phosphate may weaken bones. A thorough review by the COT (2004a,b) revealed that there is insufficient scientific literature upon which to base the assertion that phosphorus may have a bone weakening effect. Furthermore, the increase of PTH reported in some studies following phosphate administration was suggested by the COT (2004a) to be a short-term homeostatic effect to maintain the plasma ratio of calcium and phosphorus, which was not necessarily adverse. The potential long-term effects of phosphate administration remain unknown. As a result of the COT review the advisory statement regarding the potential adverse effect of phosphorus on bones was removed.

XIII.5 Potential Allergenicity Concerns

There are no potential allergy concerns with RS4-fibre*. The product is manufactured from high amylose maize and is therefore free from gluten. “Gluten Free” is described as “not consisting of or containing as ingredients such cereals as wheat, triticale, rye, barley or oats or their constituents”.[3]

Similarly, there are no potential allergy concerns with phosphorus from phosphated distarch phosphate; however, there are identified groups that are sensitive to phosphorus intake. These groups include: osteoperotic women and individuals with hypovitaminosis D (EVM, 2003; COT, 2004a,b). Excessive supplemental intake of phosphorus by these groups may exacerbate pre-existing disease conditions.

XIII.6 Summary

Phosphated distarch phosphate is metabolised like any other starch; however, the *in vitro* digestibility of raw phosphated distarch phosphate is somewhat reduced compared to unmodified starch, while the *in vivo* digestibility is relatively similar to raw unmodified

[3] Codex Standard for “Gluten-Free Foods”, Codex Stan. 118-1981 (amended 1983) Codex Alimentarius Commission.

starch (Kohn and Kay, 1963a,b). Upon processing (cooking) RS4-fibre* (phosphated distarch phosphate) maintains its resistance to digestion to a much greater degree than processed unmodified starch as depicted with the digestion curves in Figure XI.1-1. The digestibility and utilization of modified starch is similar to unmodified starch; however, the higher amount of amylose and the degree of cross-linking of RS4-fibre* increases its resistance to digestive enzymes and thus reduces its breakdown. The phosphate moiety in starch phosphate acts in a similar fashion compared to orthophosphate and pyrophosphate (Laboratories of International Minerals & Chemicals Co., 1955). Phosphorus, primarily occurring as inorganic phosphate in the diet is mainly absorbed in the jejunum *via* passive, concentration dependent processes although a portion of absorbed phosphorus is absorbed under saturable active transport facilitated by 1,25-dihydroxyvitamin D (IOM, 1997). The level of phosphorus and consequently the level of calcium in the blood is regulated by parathyroid hormone (PTH) with the level of phosphorus in the blood and the urine altered with dietary intake of calcium and phosphorus. Phosphorus is predominantly stored in the bones with a lesser amount present in the blood as a constituent of phospholipids, and to a lesser extent, inorganic phosphate. Excretion *via* the urine is the main route for the removal of phosphorus, with minor amounts lost in shed epithelial cells (*e.g.*, keratinized epithelial cells and gastrointestinal mucosal epithelial cells).

No information is currently available regarding the acute toxicity of phosphated distarch phosphate; however, studies conducted with distarch phosphate have indicated that distarch phosphate is essentially non-toxic (*i.e.*, LD_{50} values >7,000 mg/kg body weight) (Hodge, 1954, 1956). Alternately, the acute toxicity of phosphorus-containing compounds (*i.e.*, phosphoric acid, phosphates, orthophosphates, diphosphates, triphosphates, and polyphosphates) ranged from 1,300 to 4,600 mg/kg body weight (van Each *et al.*, 1957; FDRLI, 1975a,b,c,d).

In subchronic, chronic, and developmental/reproductive studies there were no adverse effects reported that were related to the test substance (*i.e.*, phosphated distarch phosphate or distarch phosphate) (Cervenka and Kay, 1963; Kohn *et al.*, 1964a,b; de Groot and Spanjers, 1970; Til *et al.*, 1970; de Knecht-Van Eekelen, 1971; Anderson *et al.*, 1973; de Groot *et al.*, 1974); however, caecal enlargement and deaths were reported (Cervenka and Kay, 1963; Kohn *et al.*, 1964a,b; de Groot and Spanjers, 1970). The development of caecal enlargement within the animals was not viewed as a toxicological effect, but rather a physiological adaptation to the consumption of slowly digestible carbohydrates (Roe, 1979). Deaths were reported in subchronic studies for both control and treatment animals; however, the deaths were unrelated to the test substance. Furthermore, PN was reported in the chronic study using phosphated distarch phosphate. Similarly, subchronic and chronic studies with phosphorus-containing compounds were reported to result in decreased growth, the occurrence of PN, and kidney damage (calcification, tubular tissue damage) (Hodge, 1956; Hahn, 1956, 1958; van Each *et al.*, 1957; Dymsza *et al.*, 1959; Hahn and Seifen, 1959; Lang, 1959; Hodge, 1960a,b;

Datta *et al.*, 1962; Ellinger, 1972; Fishwick, 1974; De Luca *et al.*, 1976; Anderson *et al.*, 1977; Clawson and Armstrong, 1981); however, no treatment-related deaths were reported. The occurrence of lesions in de Knecht-Van Eekelen *et al.* (1971) were considered by the study authors to be of no toxicological significance. Furthermore, in an extensive review of PN, Roe (1979) concluded that the occurrence of PN in laboratory animals results due to mineral imbalances and are relatively common in untreated laboratory animals, particularly older animals. The remaining adverse effects reported in studies with phosphorus containing compounds can be attributed to the induced mineral imbalance with the higher intake level of phosphorus. In developmental and reproductive toxicity studies with phosphorus-containing compounds no maternal or teratogenic effects were reported with doses ranging from 138 to 410 mg/kg body weight and 130 to 465 mg/kg body weight in rats and mice, respectively (FDRLI, 1975a,b,c,d). Phosphorus-containing compounds also tested negative for mutagenicity using *in vivo* and *in vitro* tests (Litton Bionetics, 1974, 1975a,b,c; Newell *et al.*, 1974; FDRLI, 1975d).

Additionally, phosphated distarch phosphate was reported to be well tolerated by human volunteers without the occurrence of any adverse effects (Pieters *et al.*, 1971). Supplementation with phosphorus containing compounds was generally well-tolerated (Malm, 1953; Lang, 1959; Bernstein and Newton, 1966; Goldsmith *et al.*, 1968,1976; Bell *et al.*, 1977; Brixen *et al.*, 1992; Whybro *et al.*, 1998; Grimm *et al.*, 2001); however, gastrointestinal incidents (*i.e.*, dyspepsia, nausea, vomiting, loose stools, and diarrhoea) were the main adverse effects reported in relation to the consumption of phosphorus-containing compounds. Isolated incidents of pedal and pretibial oedema, non-significant increases in serum PTH, and increases in the number of bone resorbing surfaces also were reported (Goldsmith *et al.*, 1968, 1976; Brixen *et al.*, 1992; Whybro *et al.*, 1998). The majority of the adverse effects subsided with the discontinuation of the treatment regimen. The increase in the number of bone resorbing surfaces was only reported in post-menopausal women, indicating that post-menopausal women may be a population group sensitive to mineral imbalances through the intake of high amounts of phosphorous-containing compounds.

EVALUATIONS AND CONCLUSIONS

RS4-fibre* is produced from high amylose maize through a combination of treatments designed to reduce the digestibility of the modified starch. High amylose maize is used in the production of RS4-fibre* and is permitted at a maximum residual phosphate level of 0.4% (as phosphorus).

National Starch intends to include RS4-fibre* in conventional foods as a resistant dietary fibre. RS4-fibre* will be added to cereals and cereal products (*e.g.*, biscuits, crackers, cakes and muffins, pasta, pizza dough, ready-to-eat breakfast cereals, tortillas, and bread products made with white flour), as well as crisps and savoury snacks (*e.g.*, pretzels). RS4-fibre* is an easy to use, palatable and economical dietary fibre. This can enable food manufacturers to cost-effectively increase the fibre content of everyday foods without a decrease in eating quality.

The highest intake (all-user -which would represent the worst-case over estimate) of RS4-fibre* from the proposed food categories in the U.K. is expected to occur in adult males with a 97.5th percentile intake of 72.2 g/person/day of RS4-fibre*. In addition to the estimated exposure of population groups to RS4-fibre* from the proposed food categories, the exposure to phosphorus [*i.e.*, resulting from residual (bound) phosphorus absorption] was estimated with adult males expected to be exposed to the highest level of phosphorus with a 97.5th percentile all-user intake of 288.7 mg/person/day.

The intake of RS4-fibre* and phosphorus at these levels are expected to be well-tolerated and safe as demonstrated in a tolerance study in human volunteers (Pieters *et al.*, 1971). Whilst only the 90^{th}+ percentile intakes of phosphorus in the diet of male teenagers and male adults slightly exceeds the proposed guidance level for supplemental intake of phosphorus of 250 mg/day determined by the EVM (2003), mean consumption is well below the guidance level at approximately 180 mg per day. The estimated maximum daily intake of phosphorous from the diet and additional supplements was reported to be 3,200 mg/day (*i.e.*, 1,260 or 2,110 mg/day from the mean and 97.5th percentile intake from food, respectively, and approximately 1,100 mg/day from supplements). The intake of phosphorus of the teenage males is well below the estimated average requirement (EAR) and recommended dietary allowances (RDA) determined by the IOM (1997) or 1,055 and 1,250 mg/day for male's aged 9 through 18 years, respectively. The tolerable upper intake level (UL) was 3.0 and 4.0 g for children and adults, respectively. Although the risk assessment methods differ between the IOM and the EVM, the guidance level established by the EVM is a conservative estimate of the level of intake of supplemental phosphorus not likely to result in any adverse effect and it likely to over-estimate the intake of supplemental forms of phosphorus by the population. The key studies cited by the EVM (2003) report the administration of phosphorus in capsule or solution form resulting in mild gastrointestinal side effects. The consumption of

phosphorus from the use of RS4-fibre* in a food matrix will lessen the risk of gastrointestinal side effects due to physical and dietary factors affecting phosphorus absorption in the small intestine. Additionally, the intake of phosphorous from RS4-fibre* will not result from a single bolus ingestion as in the key studies cited by the EVM, but rather from the ingestion of various food products throughout the day. Grimm *et al.* (2001) suspected that the reported gastrointestinal side effects reported were the result of the osmotic effect of phosphorus in the intestinal lumen. The presence of other dietary nutrients and physical factors may reduce the osmotic effect of phosphorus and its absorption, thereby reducing its mild gastrointestinal side effects.

In conclusion, there is a substantial body of evidence to support the safety of RS4-fibre*, a novel food ingredient based on its lack of prior history of use in the European Community. The replacement of unmodified starches or other modified starches (*i.e.*, not RS4-fibre*) in the individual proposed food uses will not expose the general population to increased risks due to additional supplemental phosphorus intake. On the basis of the available toxicology data, its nutritional equivalence to unmodified starch, and the level of phosphate intake (as phosphorus), it is concluded that RS4-fibre* does not present a significant risk for human health at the intake, which would result from its intended uses in food.

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