ORIGINAL SUBMISSION

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OFFICE OF
FOOD ADDITIVE SAFETY

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

GENERALLY RECOGNIZED AS SAFE (GRAS) NOTICE

#615

Form Approved: OMB No. 0910-0342; Expiration Date: 02/29/2016
(See last page for OMB Statement)

FDA USE ONLY

GRN NUMBER 000515	DATE OF RECEIPT
ESTIMATED DAILY INTAKE	INTENDED USE FOR INTERNET
NAME FOR INTERNET	
KEYWORDS	

Transmit completed form and attachments electronically via the Electronic Submission Gateway *(see Instructions)*; OR Transmit completed form and attachments in paper format or on physical media to: Office of Food Additive Safety *(HFS-200)*, Center for Food Safety and Applied Nutrition, Food and Drug Administration, 5100 Paint Branch Pkwy., College Park, MD 20740-3835.

PART I – INTRODUCTORY INFORMATION ABOUT THE SUBMISSION

1. Type of Submission *(Check one)*
☒ New ☐ Amendment to GRN No._____ ☐ Supplement to GRN No. _____

2. ☒ All electronic files included in this submission have been checked and found to be virus free. *(Check box to verify)*

3a. For New Submissions Only: Most recent presubmission meeting *(if any)* with FDA on the subject substance *(yyyy/mm/dd)*: N/A

3b. For Amendments or Supplements: Is your amendment or supplement submitted in response to a communication from FDA? *(Check one)* ☐ Yes ☐ No If yes, enter the date of communication *(yyyy/mm/dd)*: _____

PART II – INFORMATION ABOUT THE NOTIFIER

1a. Notifier

Name of Contact Person	Position
Henry J. Binder, MD	Professor Emeritus of and Senior Research Scientist in

Company *(if applicable)*: Yale University, Yale School of Medicine

Mailing Address *(number and street)*: LMP 1080

City	State or Province	Zip Code/Postal Code	Country
New Haven	Connecticut	06519	United States of America

Telephone Number	Fax Number	E-Mail Address
203-785-4796		

1b. Agent or Attorney *(if applicable)*

Name of Contact Person	Position

Company *(if applicable)*:

Mailing Address *(number and street)*:

City	State or Province	Zip Code/Postal Code	Country

Telephone Number	Fax Number	E-Mail Address

PART III – GENERAL ADMINISTRATIVE INFORMATION

1. Name of Substance

HAMS

2. Submission Format: *(Check appropriate box(es))*

☐ Electronic Submission Gateway

☐ Electronic files on physical media with paper signature page

☒ Paper

If applicable give number and type of physical media

_____

3. For paper submissions only:

Number of volumes _____

Total number of pages _____

4. Does this submission incorporate any information in FDA's files by reference? *(Check one)*

☐ Yes *(Proceed to Item 5)* ☒ No *(Proceed to Item 6)*

5. The submission incorporates by reference information from a previous submission to FDA as indicated below *(Check all that apply)*

☐ a) GRAS Notice No. GRN _____

☐ b) GRAS Affirmation Petition No. GRP _____

☐ c) Food Additive Petition No. FAP _____

☐ d) Food Master File No. FMF _____

☐ e) Other or Additional *(describe or enter information as above)* _____

6. Statutory basis for determination of GRAS status *(Check one)*

☐ Scientific Procedures *(21 CFR 170.30(b))* ☒ Experience based on common use in food *(21 CFR 170.30(c))*

7. Does the submission (including information that you are incorporating by reference) contain information that you view as trade secret or as confidential commercial or financial information?

☐ Yes *(Proceed to Item 8)*

☒ No *(Proceed to Part IV)*

8. Have you designated information in your submission that you view as trade secret or as confidential commercial or financial information *(Check all that apply)*

☐ Yes, see attached Designation of Confidential Information

☐ Yes, information is designated at the place where it occurs in the submission

☐ No

9. Have you attached a redacted copy of some or all of the submission? *(Check one)*

☐ Yes, a redacted copy of the complete submission

☐ Yes, a redacted copy of part(s) of the submission

☐ No

PART IV – INTENDED USE

1. Describe the intended use of the notified substance including the foods in which the substance will be used, the levels of use in such foods, the purpose for which the substance will be used, and any special population that will consume the substance *(e.g., when a substance would be an ingredient in infant formula, identify infants as a special population).*

The ingredient is being used as an ingredient.

2. Does the intended use of the notified substance include any use in meat, meat food product, poultry product, or egg product? *(Check one)*

☐ Yes ☒ No

PART V – IDENTITY

1. Information about the Identity of the Substance

	Name of Substance[1]	Registry Used *(CAS, EC)*	Registry No.[2]	Biological Source *(if applicable)*	Substance Category *(FOR FDA USE ONLY)*
1	HAMS , High Amylose Maize Starch				
1					
1					

[1] Include chemical name or common name. Put synonyms *(whether chemical name, other scientific name, or common name)* for each respective item (1 - 3) in Item 3 of Part V *(synonyms)*

[2] Registry used e.g., CAS *(Chemical Abstracts Service)* and EC *(Refers to Enzyme Commission of the International Union of Biochemistry (IUB), now carried out by the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (IUBMB))*

2. Description

Provide additional information to identify the notified substance*(s)*, which may include chemical formula*(s)*, empirical formula*(s)*, structural formula*(s)*, quantitative composition, characteristic properties *(such as molecular weight(s))*, and general composition of the substance. For substances from biological sources, you should include scientific information sufficient to identify the source *(e.g., genus, species, variety, strain, part of a plant source (such as roots or leaves), and organ or tissue of an animal source)*, and include any known toxicants that could be in the source.

control 1: 50g of rice flour
control 2: 50g high amylose maize stach
control 3: amylase-resistant starch

3. Synonyms

Provide as available or relevant:

1	HAMS
1	
1	

Add Continuation Page

PART VI – OTHER ELEMENTS IN YOUR GRAS NOTICE

(check list to help ensure your submission is complete – check all that apply)

- ☐ Any additional information about identity not covered in Part V of this form
- ☐ Method of Manufacture
- ☐ Specifications for food-grade material
- ☐ Information about dietary exposure
- ☐ Information about any self-limiting levels of use *(which may include a statement that the intended use of the notified substance is not-self-limiting)*
- ☐ Use in food before 1958 *(which may include a statement that there is no information about use of the notified substance in food prior to 1958)*
- ☐ Comprehensive discussion of the basis for the determination of GRAS status
- ☐ Bibliography

Other Information

Did you include any other information that you want FDA to consider in evaluating your GRAS notice?

☒ Yes ☐ No

Did you include this other information in the list of attachments?

☒ Yes ☐ No

PART VII – SIGNATURE

1. The undersigned is informing FDA that _____ *(name of notifier)*

has concluded that the intended use*(s)* of HAMS *(name of notified substance)*

described on this form, as discussed in the attached notice, is (are) exempt from the premarket approval requirements of section 409 of the Federal Food, Drug, and Cosmetic Act because the intended use(s) is (are) generally recognized as safe.

2. ☐ _____ *(name of notifier)* agrees to make the data and information that are the basis for the determination of GRAS status available to FDA if FDA asks to see them.

_____ *(name of notifier)* agrees to allow FDA to review and copy these data and information during customary business hours at the following location if FDA asks to do so.

_____ *(address of notifier or other location)*

_____ *(name of notifier)* agrees to send these data and information to FDA if FDA asks to do so.

OR

☐ The complete record that supports the determination of GRAS status is available to FDA in the submitted notice and in GRP No. _____ *(GRAS Affirmation Petition No.)*

3. Signature of Responsible Official, Agent, or Attorney	**Printed Name and Title**	**Date (mm/dd/yyyy)**
(b) (6)	SM Girvin Deputy Provost	12/07/2015

PART VIII – LIST OF ATTACHMENTS

List your attached files or documents containing your submission, forms, amendments or supplements, and other pertinent information. Clearly identify the attachment with appropriate descriptive file names (or titles for paper documents), preferably as suggested in the guidance associated with this form. Number your attachments consecutively. When submitting paper documents, enter the inclusive page numbers of each portion of the document below.

Attachment Number	Attachment Name	Folder Location (select from menu) (Page Number(s) for paper Copy Only)
1	Insert / Clear (*) Paper copy already sent; on file; CD enclosed with copies per pre-reviewer Richard Bonnette.	
	Insert / Clear	
	Insert / Clear	
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Add Continuation Page

OMB Statement: Public reporting burden for this collection of information is estimated to average 150 hours per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to: Department of Health and Human Services,Food and Drug Administration, Office of Chief Information Officer, 1350 Piccard Drive, Room 400, Rockville, MD 20850. (Please do NOT return the form to this address.). An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a currently valid OMB control number.

Documentation Supporting the Determination that High-Amylose Maize Starch (HAMS) is Generally Recognized as Safe (GRAS) for Use as an Ingredient in Oral Rehydration Solutions that are Medical Foods

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March 25, 2015

Documentation Supporting the Determination that High-Amylose Maize Starch (HAMS) is Generally Recognized as Safe (GRAS) for Use as an Ingredient in Oral Rehydration Solutions that are Medical Foods

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Documentation Supporting the Determination that High-Amylose Maize Starch (HAMS) is Generally Recognized as Safe (GRAS) for Use as an Ingredient in Oral Rehydration Solutions that are Medical Foods

1.0 INTRODUCTION

Yale University (Yale) intends to market a new oral rehydration solution (ORS) that contains resistant starch, specifically high-amylose maize starch (HAMS), as a replacement for glucose. Hypo-osmolar glucose-electrolyte based solutions are currently endorsed by various authoritative bodies, such as the World Health Organization (WHO), United Nations Children's Fund (UNICEF), and the European Society for Paediatric Gastroenterology, Hepatology, and Nutrition and European Society of Paediatric Infectious Diseases (ESPGHAN-ESPID), for the management of dehydration associated with diarrhea (UNICEF, 2003; WHO/UNICEF, 2006; Guarino *et al.*, 2008). In the United States (U.S.), ORS products are regulated as medical foods under the Federal Food, Drug, and Cosmetic Act (U.S. FDA, 2013a). Although HAMS is a widely accepted food ingredient that is permitted for use in most countries without any limitations on its use, it has not been commonly used for nutritive purposes in foods prior to 1958. Therefore, the use of HAMS as the sole carbohydrate source in ORS will need to be determined as Generally Recognized as Safe (GRAS) based on scientific procedures.

The purpose of this dossier is to (i) outline the identity and manufacture of HAMS, (ii) define the intended conditions of use and use levels of the ingredient in ORS, (iii) estimate exposure under the conditions of intended use, and (iv) document the literature pertaining to the safety of HAMS when used in ORS. To obtain the necessary information, comprehensive and detailed searches of the published scientific literature were conducted by Intertek Scientific & Regulatory Consultancy through February 2014. MedLine, ToxFile, Agricola, Agris, Allied and Complementary Medicine, Biosis ToxLine, Foodline: Science, CAB Abstracts, FSTA, NTIS, Embase, and Adis Clinical Trials served as the primary sources of published literature pertinent to the safety of HAMS as a replacement for glucose in ORS. Information pertaining to the composition and manufacturing process for HAMS, as well as batch analyses and intended uses are presented.

The data and information summarized in this dossier demonstrate that HAMS, produced using current Good Manufacturing Practices (cGMP) and meeting appropriate food-grade specifications, is GRAS under the conditions of intended use in ORS based on scientific procedures, as described herein.

2.0 DESCRIPTION OF HIGH-AMYLOSE MAIZE STARCH (HAMS)

2.1 Common or Usual Name

High-amylose maize starch; high-amylose corn starch

2.2 Trade Name

Not applicable

2.3 Chemical Name

Not applicable.

2.4 Chemical Abstract Service (CAS) Number

The CAS number for starches is 9005-25-8.

2.5 Physical and Chemical Characteristics

2.5.1 Physical Properties

In general, unmodified food starches are insoluble in alcohol, ether and chloroform (FCC, 2014). They are also insoluble in cold water, unless they are treated with to be pregelatinized or made cold-water swelling (FCC, 2014). When heated in water, the starch granules usually begin to swell at temperature between 45 and 80°C, and gelatinize completely at higher temperatures (*i.e.*, form colloidal solutions with viscous properties) (FCC, 2014). However, HAMS is resistant to gelatinization, with complete gelatinization not occurring until temperatures of 154 to 171°C, which is higher than the temperatures that are typically encountered in food processing (Brown, 1994). When cooked, HAMS paste is very viscous, and it has a strong tendency to retrograde (White, 2001). Unmodified food starches have technological functions as thickener, colloidal stabilizer, and binder (FCC, 2014).

2.5.2 Chemical Properties

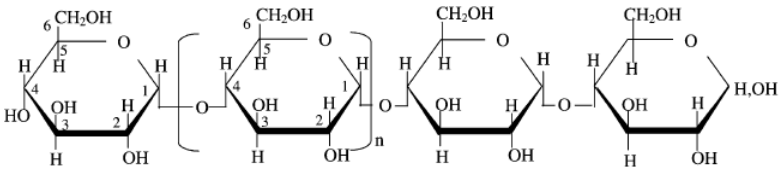
Starches are polysaccharides, and they are the major dietary source of carbohydrates (Sajilata *et al.*, 2006). There are 2 main structural components of starch; amylose is a linear polymer of glucose residues bound *via* α-D-(1,4)-glycosidic linkages, while amylopectin is a highly branched molecule comprising α-D-(1,4)-linked glucopyranose units with α-D-(1,6)-glycosidic branch points (Sajilata *et al.*, 2006). Branch points typically occur between chain lengths of 20 to 25 glucose units, and account for approximately 5% of the glycosidic linkages (Sajilata *et al.*,

2006; Jiang and Jane, 2013). Normal maize starch (from *Zea mays* L.) typically consists of approximately 25 to 30% amylose and 75 to 80% amylopectin (Wang *et al.*, 1993; Jiang and Jane, 2013). However, through traditional breeding programs that select for genetic variants of maize with a high amylose content, hybrid maize known as amylomaize have been developed that can yield starch (*i.e.*, HAMS) containing 55 to >90% amylose (BeMiller, 1973; Richardson *et al.*, 2000; Sajilata *et al.*, 2006).

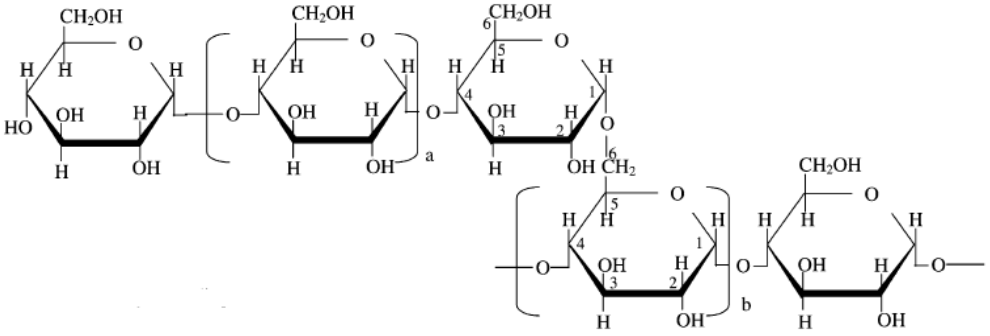
Molecular Formula: $(C_6H_{10}O_5)_n$

Structural Formula:

A) Amylose (Average DP for HAMS: 500)



B) Amylopectin (Average DP: 2 million)



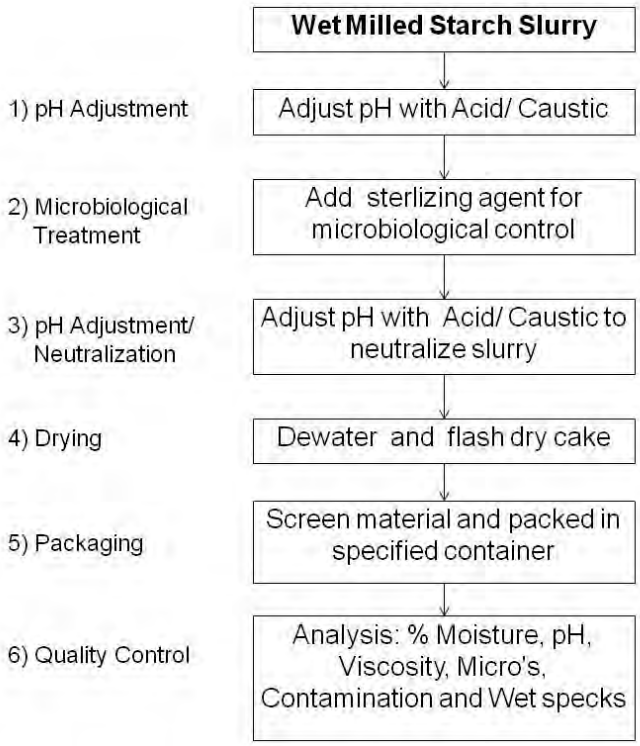
3.0 METHOD OF MANUFACTURE

3.1 Manufacturing Process

The manufacturing process of HAMS (Hylon-VII®), as highlighted in Figure 3.1-1, is similar to those commonly employed in the industry to manufacture cornstarch (U.S. EPA, 1994). Dehulled corn kernels undergo wet-milling to produce a starch slurry. The starch slurry is then dried and packaged, and quality control analyses are conducted. The HAMS (Hylon-VII®) ingredient intended for use in ORS is manufactured in accordance with cGMP, and it has been

marketed as a food ingredient (*e.g.*, for its gelling, film forming and opacifying properties) in the U.S. and globally for many years.

Figure 3.1-1 Schematic Overview of the Manufacturing Process for High-Amylose Maize Starch (HAMS)



3.2 Product Specifications

The HAMS (Hylon-VII®) intended for use in ORS meets the specifications for food-grade unmodified starches defined in the Food Chemicals Codex, as presented in Table 3.2-1.

Table 3.2.1-1 Food-Grade Specifications for Unmodified Food Starches in the Food Chemicals Codex (FCC, 2014)

Parameter	Specification
Identity	
Iodine stain	Positive (dark blue to red color)
Copper reduction	Copious red precipitate forms
Microscopy	Typical polarization cross
Crude fat (%)	NMT 0.15
Protein (%)	NMT 1 for high-amylose-starch
Loss on drying (%)	NMT 15.0
pH	3.0 to 9.0

Table 3.2.1-1 Food-Grade Specifications for Unmodified Food Starches in the Food Chemicals Codex (FCC, 2014)	
Parameter	**Specification**
Lead (mg/kg)	NMT 1
Sulfur dioxide (mg/kg)	NMT 50

NMT = not more than

3.3 Batch Analysis

Analysis of 5 non-consecutive lots of HAMS (Hylon-VII®) demonstrates that the manufacturing process produces a consistent product that is free from microbial and heavy metal contamination. A summary of the batch analysis data for HAMS (Hylon-VII®) is presented in Table 3.3-1 (see Appendix A for Certificates of Analysis).

Table 3.3-1 Batch Analysis Data for 5 Non-Consecutive Lots of High-Amylose Maize Starch (HAMS)

Parameter	Manufacturing Lot				
	JCK3327	**ACK5040**	**CCK5123**	**MCK3418**	**ECK3151**
pH	5.51	5.17	5.96	5.60	5.72
Moisture (%)	11.5	12.70	11.76	12.16	12.24
Heavy Metals					
Lead (mg/kg)	0.00614	<0.005	<0.005	0.0301	Not conducted
Microbial Contamination					
Total plate count	130	140	210	30	50
Yeast	0	<10	<10	0	0
Mold	0	<10	<10	20	0
Escherichia coli	Negative	Negative	Negative	Negative	Negative
Salmonella	Negative	Negative	Negative	Negative	Negative
NFPA[a]	Pass	Pass	Pass	Pass	Pass

[a] National Food Processors Association standards for spore-forming bacteria in sugar and starches

3.4 Resistant Starch Content

It is well-recognized that a variable, but substantial, proportion of ingested starch resists digestion and absorption in the upper gastrointestinal tract (Cummings and Englyst, 1995; Topping and Clifton, 2001; Sajilata *et al.*, 2006). These non-digestible starch fractions are known as resistant starches (RS). By definition, resistant starch refers to the sum of starch and products of starch degradation that are not absorbed in the small intestines of healthy individuals (Asp and Björk, 1992; Jiang and Jane, 2013). Resistant starches have been classified into different types according to the mechanism by which they resist digestion. High-amylose maize starch, along with starches from other dietary sources (such as raw potato,

green banana, and certain legumes), belong to type 2 (RS_2), which are considered to be naturally occurring starches that are resistant to enzyme digestion because of their specific granular structure (Brown, 1994; Topping *et al.*, 2003; Sajilata *et al.*, 2006). Starches that have been chemically modified to be resistant to digestion (*e.g.*, by esterification, etherization, or cross-linking) are classified as Type 4 resistant starch (RS_4) (Brown, 1994; Topping *et al.*, 2003; Sajilata *et al.*, 2006; Slizewska *et al*., 2012). Other types of resistant starches include those that are physically protected and inaccessible to digestion, such as whole or partly-milled seeds and grains (RS_1); indigestible starch that is formed by retrogradation upon gelatinization (RS_3); and amylose-lipid complexed starch (RS_5) (Brown, 1994; Topping *et al.*, 2003; Sajilata *et al.*, 2006; Slizewska *et al*., 2012).

The resistant starch content of HAMS (Hylon-VII®) has been estimated at 73 to 75% (dry weight basis) using the Englyst digestion method.

3.5 Stability

In general, starch is known to be an inert compound that is relatively stable when stored in its bulk form (*i.e.*, dry powder) for indefinite periods of time, as long as it is kept dry. Although formal stability studies have not been conducted with HAMS (Hylon-VII®), a shelf-life of 2 years has been established on the basis of its low water activity and generally accepted storage stability (see letter provided by supplier in Appendix B). It is notable that HAMS (Hylon-VII®) is likely to be stable for longer than 2 years, but deterioration of the packaging material may potentially compromise the stability of the starch within. As support of the long-term bulk stability of HAMS, there were no notable changes in the chemical or microbiological parameters of a manufacturing batch of Hylon-VII® that was kept for 3 years (Table 3.5-1).

Table 3.5-1 Stability of High Amylose Maize Starch (Hylon-VII®) when Stored as Bulk Powder Under Recommended Conditions for 3 Years		
Parameter	**Lot CH4115 (March 2007)**	**Lot CH4115 (March 2010)**
pH	5.9	5.8
% Moisture	10.8	14.3
Wet specks	Pass	Pass
Odor	Pass	Pass
KI	Negative	Negative
Viscosity	87.0	78.3
SO_2	0.0	0.0
Protein	0.69	0.55
% Amylose	68.0	68.2
% Contamination	2.3	1.7
Microbial Contamination		
Total plate count	10	42
Escherichia coli	Negative	Negative
Salmonella	Negative	Negative
Yeast	1	1
Mold	1	1
NFPA[a]	Pass	Pass

[a] National Food Processors Association standards for spore-forming bacteria in sugar and starches

4.0 INTENDED USE OF HIGH-AMYLOSE MAIZE STARCH (HAMS)

4.1 Current Regulatory Status

4.1.1 High-Amylose Maize Starch

Overall, food starches (including HAMS) have been used in the food supply for many years across the globe. Varieties of amylomaize have been available since the 1950s, and commercial preparations of HAMS have been consumed since then as food ingredients (BeMiller, 2009). Furthermore, HAMS serves as the source material for the production of chemically modified food starches, which also have an extensive history of use in the food supply (BeMiller, 2009). The Joint FAO/WHO Expert Committee on Food Additives (JECFA) has indicated that native food starches, amylose and amylopectin, should be considered as foods/food ingredients rather than food additives (JECFA, 1969, 1974). The Acceptable Daily Intake (ADI) level was derived as “not specified”, a designation that is given to ingredients that are of very low toxicity and that JECFA do not consider to pose a hazard to human health at the total dietary intake of the substance arising from its use at the levels necessary to achieve the desired effect and from its background intake in foods (JECFA, 1974, 1982).

In the U.S., the Select Committee on GRAS Substances (SCOGS) has evaluated the safety of various types of starches, including HAMS. It was concluded that "*there is no evidence in the available information on unmodified or pregelatinized corn, high amylose corn, waxy maize, wheat, milo (also called grain sorghum starch), rice, potato, tapioca or arrowroot starch that demonstrates or suggests reasonable grounds to suspect a hazard to the public when they are used at levels that are now current or that might reasonably be expected in the future*" (FASEB, 1979). On this basis, the U.S. Food and Drug Administration (FDA) drafted a proposed rule (50 FR 12821 – U.S. FDA, 1985) to amend Part 184 (Direct Food Substances Affirmed as Generally Recognized as Safe) of Title 21 of the Code of Federal Regulations to include unmodified food starches (21 CFR §184.1847). Under this proposed rule, unmodified food starches, including high-amylose corn starch, are considered GRAS for use in foods with no limitations other than cGMP (50 FR 12821 - U.S. FDA, 1985). This proposed rule, along with several other proposed GRAS actions listed in the Notice of Intent, were ultimately withdrawn due to the large backlog of pending proposals and limited resources of the FDA to adequately review the comments and take action in a timely manner (69 FR 68831 – U.S. FDA, 2004) . Nevertheless, the FDA has indicated that withdrawal of these proposed rules does not affect the regulatory status of the ingredients listed in these documents (69 FR 68831 - U.S. FDA, 2004).

Starch (unmodified) and cornstarch specifically are considered GRAS as substances migrating to food from paper and paperboard products used in food packaging (21 CFR 182.90) (U.S. FDA, 2013b). Cornstarch, potato starch, tapioca starch, and wheat starch are also considered GRAS substances migrating from cotton and cotton fabrics used in dry food packaging (21 CFR 182.70) (U.S. FDA, 2013c).

4.1.2 Oral Rehydration Salts

Oral rehydration salts refers to a balanced glucose-electrolyte mixture that has been approved, recommended, and distributed by WHO and UNICEF since 1969 for the management of clinical dehydration across the world (WHO/UNICEF, 2006). The WHO/UNICEF endorsed oral rehydration salts, which can be formulated as a powder, tablet, or liquid, is classified as a drug and is treated as such in most countries worldwide (WHO, 2005; WHO/UNICEF, 2006). However, since ORS are not efficacious in treating diarrhea (*i.e.*, reducing stool output or the duration of diarrhea), but rather addresses the dehydration and metabolic acidosis that are a consequence of diarrhea (Binder *et al.*, 2014), ORS can be considered as a form of nutritional support during episodes of diarrhea, and they are classified as medical foods in the U.S. (U.S. FDA, 2006).

Although the WHO/UNICEF endorsed ORS formulation specifies the use of food-grade anhydrous glucose (dextrose), ORS containing various forms of cereals (*e.g.,* rice, wheat, maize, sorghum, and millet) as a replacement for glucose have also been used in the management of dehydration during acute diarrhea worldwide for many years (WHO, 2005;

WHO/UNICEF, 2006). There is evidence to suggest that rice-based ORS may be superior to standard glucose-based ORS, particularly in the management of cholera diarrhea in adults and children, and some authoritative bodies have indicated that rice-based ORS may be recommended for these cases (Guarino *et al.*, 2008; WGO, 2012).

According to proposed rule on the Regulations of Medical Foods (61 FR 60661 – U.S. FDA, 1996), ORS are considered to be solutions of water, electrolytes, and a carbohydrate source. There are no specific limitations on the ingredients that may be used to fulfill the requirement of a carbohydrate source, or the amount of electrolyte that must be present. A number of ORS formulations are currently marketed in the U.S. (*e.g.,* Pedialyte®, Naturalyte®, Enfalyte®, and CeraLyte®) that are intended to help replace the water and salts lost during acute diarrhea in children and adults (U.S. FDA, 2011). There is no prior history of HAMS or other types of resistant starches being used as a carbohydrate source in ORS in the U.S., though rice-based ORS (*e.g.,* CeraLyte®) are currently marketed.

4.2 Intended Use of HAMS and Levels of Use

4.2.1 Management of Dehydration During Acute Diarrhea

The WHO defines diarrhea as "*the passage of unusually loose or watery stools, usually at least 3 times in a 24-hour period. However, it is the consistency rather than the number that is most important"* (WHO, 2005). Acute watery diarrhea is one of the main clinical forms of diarrhea, and it is associated with significant fluid loss and rapid dehydration (WHO, 2005; UNICEF/WHO, 2009). Acute diarrhea typically lasts less than 7 days, and not longer than 14 days (Guarino *et al.*, 2008). In industrialized countries, acute diarrhea is often caused by viral infections (*e.g.*, by rotavirus, or human caliciviruses such as norovirus), whereas infections by enteric bacteria (*e.g.*, enterotoxigenic *Escherichia coli*, *Vibrio cholera* O1 or O139, *Campylobacter*, *Shigella* species, and Salmonella) tend to be a more common cause of acute diarrhea in developing countries (WHO, 2005; WGO, 2012). Enteric pathogens cause diarrhea by altering the movement of ions and water, either directly through modulation of barrier function or ion transport processes by increasing intracellular cyclic adenosine monophosphate (cAMP) levels, or indirectly through inflammation, secretion of neuropeptides, or loss of absorptive surfaces (Hodges and Gill, 2010) (see Section 6.4).

Dehydration is the most serious consequence of diarrhea, and could lead to severe life-threatening illness if the water and electrolytes (*i.e.*, sodium, chloride, potassium, and bicarbonate) lost in the liquid stool are not quickly replaced (CDC, 2003; UNICEF/WHO, 2009). Oral rehydration solutions are a simple, cost-efficient method of replacing fluids lost from diarrhea and reducing the associated dehydration (CDC, 2003; UNICEF/WHO, 2009). These preparations enhance fluid uptake by acting on the glucose-coupled sodium transport system at the intestinal brush border; the presence of glucose in the ORS improves absorption of sodium,

which enhances the passive absorption of water as a result of the osmotic gradient generated (CDC, 2003). The functions of the transport systems within the intestinal mucosa are maintained even during diarrhea of pathogenic origins, and thus standard glucose-based ORS are suitable for use in the management of dehydration during acute diarrhea of all etiology (CDC, 2003; WHO, 2005; WGO, 2012). It has been estimated that the volume lost through stools within the first 24 hours can vary from 5 mL/kg (minimal dehydration) to over 200 mL/kg (WHO, 2005).

4.2.2 Target Population

ORS containing HAMS as a carbohydrate source is intended for use in children (>2 years of age) and adults with acute diarrhea (*i.e.*, <7 days in duration), with or without signs of mild to moderate dehydration[1], in order to replace existing and ongoing fluid losses. According to the WHO, there are nearly 1.7 billion cases of diarrheal diseases worldwide every year, and acute diarrheal diseases remain one of the leading causes of death in children under 5 years of age, particularly among developing countries (WHO, 2013). Although mortality rates from diarrhea are much lower among industrialized countries, acute gastroenteritis is still a major cause of morbidity, accounting for more 375 million episodes (average of 1.4 episodes per person per year) and 900,000 hospitalizations per year within the U.S. alone (WGO, 2008). Of these, 1.5 million outpatient visits and 200,000 hospitalizations are represented by children (CDC, 2003; WGO, 2008).

4.2.3 Recommended Use of Oral Rehydration Solutions

Unless signs of severe dehydration are evident, the management of diarrhea typically occurs at home, with commercially available ORS being administered for rehydration under the guidance of a health-care provider (CDC, 2003). A number of authoritative bodies have published guidelines on the management of acute diarrhea in clinical practice (reviewed in van den Berg and Bergen, 2011). Specifically, recommendations on the amount of ORS that should be administered during the management of acute diarrhea (from all etiologies, including cholera diarrhea) have been published by the WHO and the World Gastroenterology Organisation (WGO), Centers for Disease Control and Prevention[2] (CDC) (CDC, 2003; WHO, 2005; WGO, 2012). These recommendations are summarized in Table 4.2.3-1 below.

[1] The CDC defines “minimal to no dehydration” as <3% loss of body weight, and “mild to moderate” dehydration as 3 to 9% loss of body weight (CDC, 2003). Similarly, the WHO has indicated that there is “no signs of dehydration” if fluid deficit is <5% of body weight, and “some dehydration” is present if fluid deficit of 5 to 10% body weight is observed. The symptoms that are associated with dehydration states (*e.g.*, sunkeness of the eyes, skin pinch response, amount of urine output, thirst, mental status, heart rate and blood pressure, rate of breathing) are described further in the systematic review of authoritative guidelines of acute gastroenteritis in children that was conducted by van den Berg and Berger (2011).

[2] The American Academy of Pediatrics has also indicated that they accept and endorse the guidelines for managing acute gastroenteritis that were published by the CDC (AAP, 2004).

Table 4.2.3-1 Recommendations for the Management of Diarrheal-Associated Dehydration in Children and Adults

A) Rehydration Phase (Minimal to Moderate Dehydration)[a]			
Age group	**Amount of ORS to Administer**		
	WHO, 2005	**WGO, 2012**	**CDC, 2003**
Children (age not specified)	75 mL/kg bw over the first 4 hrs[a], providing up to 20 mL/kg bw/hr	50 to 100 mL/kg bw over the first 3 to 4 hrs	50 to 100 mL/kg bw over the first 3 to 4 hrs
Adults	75 mL/kg bw during the first 4 hrs, providing up to 750 mL/hr	ORS should be provided for rehydration. Amount not specified.	Recommendations not provided
B) Maintenance Phase			
Age group	**Amount of ORS to Administer After Each Loose Stool or Vomiting Episode**		
	WHO, 2004, 2005	**WGO, 2012**	**CDC, 2003**
Young children (<2 yrs or <10 kg)	50 to 100 mL, up to 500 mL/day	50 to 100 mL	60 to 120 mL
Older children	100 to 200 mL, up to 1L/day (2 to 10 yrs)	Amount not specified	120 to 240 mL (>10 kg)
Adults	As much as needed, up to 2 L/day	Amount not specified	Recommendations not provided

bw = body weight; ORS = oral hydration solution

[a] The recommended treatment plan for individuals with severe dehydration (who would require intravenous fluid replacement at a medical facility prior to ORS administration) is not provided here, since ORS containing HAMS is intended for use only in children and adults with mild to moderate diarrhea. Individuals with acute diarrhea, but no signs of dehydration, require only maintenance of ongoing fluid losses.

[b] If the body weight of a child is unknown, the WHO developed guidelines on the approximate volume of ORS that should be administered according to age (WHO, 2005). The recommended volumes of ORS to be consumed during the initial rehydration phase (first 4 hours), according to age, are as follows: 200 to 400 mL (<4 months); 400 to 600 mL (4 to 11 months); 600 to 800 mL (12 to 23 months); 1,200 to 2,200 mL (5 to 14 years); and 2,200 to 4,000 mL (≥15 years).

Oral rehydration therapy to correct diarrhea-associated dehydration consists of 2 phases. During the rehydration phase, water and electrolytes are administered (*e.g.,* in the form of ORS) to replace existing losses from diarrhea and possibly vomiting among individuals who exhibit at least some signs of dehydration (CDC, 2003; WGO, 2012). Oral rehydration should be performed quickly (*i.e.*, within 3 to 4 hours) (CDC, 2003). This is followed by a maintenance phase, which involves replacement of ongoing fluid and electrolyte losses to maintain continued hydration until diarrhea stops (CDC, 2003; WGO, 2012). It is important to note that the recommendations regarding the amount of ORS that should be provided are rough approximations, and the exact amount required will depend on the individual's dehydration status (WHO, 2005). Those with more marked signs of dehydration or passing frequent watery stools will require more ORS, and unless signs of over-hydration are evident (*e.g.*, puffy eyelids), the ORS can be consumed *ad libitum* (WHO, 2005).

4.2.4 Use Levels of HAMS in Oral Rehydration Solutions

Currently, the WHO/UNICEF recommended formulation of ORS consists of 4 key ingredients: glucose, sodium chloride, potassium chloride, and trisodium citrate (WHO, 2005). In 2001, the WHO and UNICEF revised the formulation of ORS to reduce the total osmolarity from 311 mOsmol/L to 245 mOsmol/L (WHO/UNICEF, 2006). This reduced osmolarity ORS formulation reduces the adverse effects of hypertonic solutions (*i.e.*, high levels of solute) on net fluid absorption while still providing an optimal ratio of glucose to sodium, and it is currently endorsed for the prevention/treatment of clinical dehydration associated with diarrhea (all etiologies) in all age groups (CDC, 2003; UNICEF, 2003). Commercially available preparations of ORS that are currently marketed in the U.S. is based on the WHO/UNICEF recommended formulation for reduced osmolarity ORS (Table 4.2.4-1). It should be noted that the ESPGHAN also recommends a "hypotonic osmolarity solution" that contains 60 mmol/L of sodium (Na^+) (Guarino *et al.*, 2008). Yale intends to add HAMS at levels of 50 g/L in ORS.

Table 4.2.4-1 Composition of the Oral Rehydration Solution Endorsed by Authoritative Bodies and Commercially Available Preparations[a]

Preparation	Carbohydrate (source)	Sodium (mmol/L)[b]	Potassium (mmol/L)[b]	Chloride (mmol/L)[b]	Citrate (mmol/L)[b]	Osmolarity (mOsm/L)
Recommendations by Authoritative Bodies						
WHO/UNICEF Iso-osmolar Formula	20 g/L or 111 mEq/L (glucose)	90	20	80	10 (30 mM base)	311
WHO/UNICEF Reduced Osmolarity Formula	13.5 g/L or 75 mEq/L (glucose)	75	20	65	10 (30 mM base)	245
WHO/UNICEF acceptable range	At least equal that of sodium but not exceed 111 mmol/L (glucose)	60 to 90	15 to 25	50 to 80	8 to 12 (24 to 36 mM base)	200 to 310
ESPGHAN Formula	16 g/L carbohydrate or 74 to 111 mmol/L (glucose)	60	20	60	10 (30 mM base)	200 to 250

Table 4.2.4-1 Composition of the Oral Rehydration Solution Endorsed by Authoritative Bodies and Commercially Available Preparations[a]

Commercially Available ORS Sold in the United States						
Brand Name	**Carbohydrate (source)**	**Sodium (mmol/L)[b]**	**Potassium (mmol/L)[b]**	**Chloride (mmol/L)[b]**	**Base (mmol/L)[b]**	**Osmolarity (mOsm/L)**
CeraLyte®	40 g/L (Rice starch/ maltodextrin)	50 to 90	20	Not applicable	30	200 to 260
Pedialyte®	25 g/L (glucose, fructose)	45	20	35	30	250
Enfalyte®	30 g/L (corn syrup solids)	50	25	45	34	200
Naturalyte®	25 g/L (glucose)	45	20	Not applicable	48	265
ORS Containing HAMS	50 g/L (HAMS)	-	-	-	-	-

HAMS = high-amylose maize starch; ORS – oral rehydration solution
[a] Data taken from ESPGAN, 1992; WHO, 2002; UNICEF, 2003; CDC, 2003; Kelly and Nadeau, 2004; Atia and Buchman, 2009
[b] The concentrations are given in mmol/L, in accordance with the International System of Units (SI). They correspond exactly to mEq/L for all salts listed with the exception of base. For example, the WHO/UNICEF endorsed solution contains trisodium citrate, where 9.86 mmol/l (rounded up to 9.9 mmol/l) citrate ($C_6H_5O_7$) corresponds to about 29.6 mEq/L of base. Other bases can also be used (*e.g.*, lactate, citrate, or acetate).

5.0 ESTIMATED INTAKE OF HAMS FROM ITS USE IN ORAL REHYDRATION SOLUTION

5.1 Background Dietary Intakes

5.1.1 High-Amylose Maize Starch

In the mid-1940s, research was conducted with the goal of developing a variety of maize with starch that consisted mainly or entirely of amylose molecules, which would be a counterpart to the already commercialized waxy maize, a spontaneous mutant of corn that produces all-amylopectin starch (BeMiller, 2009). Naturally occurring variants of corn that contained higher than normal amylose starch content were identified, and through conventional breeding programs, maize hybrids were developed that had large yields of starch with a high-amylose content (*i.e.*, amylomaize) (BeMiller, 2009). High-amylose maize hybrids first became commercially available in 1958 (BeMiller, 2009). There are currently 2 classes of amylomaize grains that are used by wet millers; the Class V hybrids produces grain containing approximately 55 to 60% amylose starch, while Class VII hybrids contain 70 to 80% amylose starch content (White, 2001). Other hybrids have been developed that produces starch with >90% amylose content (Richardson *et al.*, 2000).

The unique physical properties of HAMS have made it useful for a large variety of food applications; for example, the high gelling capacity of high amylose starches have rendered it useful in the confectionary industry as a component of candies, and as a thickener in puddings and other processed foods (Richardson *et al.*, 2000). The ability of high amylose starches to form firm, crispy and crunch films have allowed them to be used in the coating of battered food products such as french fries, frozen meats, fish, poultry and vegetables (Young, 1984; Richardson *et al.*, 2000; Hallauer, 2004). According to the review conducted by the SCOGS on the health aspects of starch and modified starches as food ingredients, it is estimated that the daily intake of unmodified starches as a component of foods in the average American diet is approximately 180 g *per capita* per day (FASEB, 1979).

5.1.2 Resistant Starches

The Institute of Medicine (IOM) of the National Academy of Sciences has developed evidence-based recommendations on the levels of various macronutrients and micronutrients that should be consumed through the diet on a daily basis (IOM, 2005). The IOM considers resistant starch that is naturally occurring and inherent in a food or created during normal processing of a food to be "dietary fibre", while isolated or extracted non-digestible carbohydrates (*e.g.*, using chemical, enzymatic, or aqueous steps) are considered as "functional fibre" (IOM, 2005). The Adequate Intake[3] for total fiber (sum of dietary fiber and functional fiber) was established to be 19 g/day for children (1 to 3 years), 25 g/day for children (4 to 8 years), 21 to 26 g/day for females (9 years and older), and 30 to 38 g/day for males (9 years and older) (IOM, 2005). Using intake data from the Continuing Survey of Food Intakes by Individuals (CSFII) (1994-1996, 1998), the IOM estimated the median dietary intake of total fiber range from 16.5 to 17.9 g/day for men and 12.1 to 13.8 g/day for women in the United States (IOM, 2005).

Several studies have also been published where the intake of resistant starches were calculated using national consumption data from dietary surveys and published literature values for resistant starch content of specific foods (Dyssler and Hoffem, 1995; Roberts *et al.*, 2004; Murphy *et al.*, 2008). Additionally, the intake of resistant starch has been estimated for diets that are considered to be representative of those that are typically consumed in India and China (Platel and Shurpalekar 1994; Muir *et al.*, 1998). These intake estimates are summarized in Table 5.1.2-1. However, it is notable that the estimated intakes of resistant starch are meant for indicative purposes only, and should not be used as detailed comparisons between countries, due to the differences in how these intakes were estimated by the different studies (*e.g.*, analytical methods used to estimate dietary content of resistant starch, and the dietary intake surveys used) (Asp *et al.*, 1996; Goldring, 2004).

[3] The IOM defines Adequate Intake as "*the recommended average daily intake level based on observed or experimentally determined approximations or estimates of nutrient intake by a group (or groups) of apparently healthy people that are assumed to be adequate*" (IOM, 2005).

Table 5.1.2-1 Estimated Dietary Intake of Resistant Starches in Various Countries		
Country	**Estimated Intake of Resistant Starch (g/day)**	**Reference**
United States	In all individuals ≥ 1 yr old[a] Mean: 4.9; Range: 2.8 to 7.9	Murphy *et al.*, 2008
Europe	In 10 different countries[b] Mean: 4.1; Range: 3 to 6	Dyssler and Hoffem, 1995
Australia/New Zealand	Minimum (Mean±SEM)[c]: 3.4±0.03 Maximum (Mean±SEM)[c]: 9.4±0.07	Roberts *et al.*, 2004
India	Mean[d]: 10	Platel and Shurpalekar, 1994
China	Mean ± SEM[e]: 18±1.1	Muir *et al.*, 1998

SEM = standard error of the mean
[a] Estimates were determined based on the content of resistant starch in foods and the reported intakes of these foods based on dietary surveys.
[b] Intakes were estimated using national consumption data from 10 different European countries. The lowest intake of resistant starch was reported in Norway (3 g/day) and the highest was reported in Spain (6 g/day).
Range depends on the lower and upper estimate of resistant starch content in foods from which intakes were calculated.
[c] Intakes were estimated using national consumption data and reported resistant starch content of individual foods. Because a range in the content of resistant starch was reported in the literature, the authors calculated the intakes using both the minimum and maximum reported values of resistant starch for the individual food categories.
[d] Estimated based on the analyzed content of resistant starch in among cereals, legumes, and vegetables, and the estimated consumption of these food groups in India.
[e] Content of resistant starch were measured in a simulated Chinese diet.

5.2 Estimated Consumption of HAMS from Uses in Oral Rehydration Solutions

As described in Section 4.2.2, a number of authoritative bodies have developed recommendations on the amount of ORS that should be consumed during the rehydration and maintenance phases among individuals with mild to moderate diarrhea (CDC, 2003; WHO, 2005; WGO, 2012). The estimated intake of HAMS that would be expected, based on the most conservative amount of ORS that should be consumed as recommended by the authoritative bodies, are summarized in Table 5.2-1.

Table 5.2-1 Intake of High-amylose Maize Starch (HAMS) Based on Recommended Uses of Oral Rehydration Solution for Managing Dehydration During Acute Diarrhea

Age	Weight (kg)	Rehydration Phase (First 4 Hours)		Maintenance Phase		Intake of HAMS in First 24 Hours (g)[d]	Intake of RS in First 24 Hours (g)
		Recommended Amount of ORS (mL)[a]	Intake of HAMS (g)[b]	Recommended Amount of ORS (mL/day)[c]	Intake of HAMS (g/day)[b]		
<4 months	<5	500	25	Up to 500	25	50	
4 to 11 months	5 to 7.9	500 to 790	25 to 40	Up to 500	25	50 to 65	
12 to 23 months	8 to 10.9	800 to 1,090	40 to 55	Up to 500	25	65 to 80	
2 to 4 years	11 to 15.9	1,100 to 1,590	55 to 80	2 to 9 years: Up to 1000	2 to 9 years: 50	105 to 250	
5 to 15 years	16 to 30	1,600 to 3,000	80 to 150	≥10 years: up to 2,000	≥10 years: 100		
>15 yrs (adults)	>30	3,000 to 4,000	150 to 200	As needed up to 2,000	100	250 to 300	

ORS = oral rehydration solution

[a] For children (≤15 years of age), ORS was assumed to be consumed at 100 mL/kg body weight for the first 4 hours of rehydration (WHO, 2005; CDC, 2003; WGO, 2012). The WHO has also provided recommendation on the specific volume of ORS to consume according the age groups, if the body weight of the individual is not available. The recommended volumes of ORS to be consumed during the initial rehydration phase (first 4 hours), according to age, are as follows: 200 to 400 mL (<4 months); 400 to 600 mL (4 to 11 months); 600 to 800 mL (12 to 23 months); 800 to 1,200 mL (2 to 4 years); 1200 to 2200 mL (5 to 14 years); and 2,200 to 4,000 mL (≥15 years). For adults, the amount of ORS consumed should provide 75 mL/kg body weight for the first 4 hours of rehydration, providing up to 750 mL/hr (*i.e.*, 3,000 mL) (WHO, 2005).

[b] HAMS will be added to ORS at 50 g/L.

[c] Recommendations by the WHO (2004, 2005).

[d] Sum of the intake of HAMS during the first 4 hours of the rehydration phase, plus intake of HAMS during the maintenance phase.

[e] HAMS contains XX% of resistant starch.

It is important to note that large amounts of ORS are likely to be consumed only during the first 24 hours of oral rehydration therapy, in order to replace the existing fluid and electrolyte losses among individuals who are exhibiting signs of mild to moderate dehydration. In individuals with diarrhea but are not yet dehydrated, or once rehydration has been achieved in dehydrated individuals, smaller amounts of ORS will be consumed as means of replacing the ongoing fluid and electrolyte losses from subsequent diarrhea/vomiting episodes. It is recommended that children 2 to 10 years of age consume 100 to 200 mL of ORS after each loose stool or vomiting episode, up to 1 L of ORS/day. Under the intended use level of HAMS (50 g/L), this would be equivalent to the consumption of 5 to 10 g HAMS per loose stool or vomiting episode, or up to 50 g/day of HAMS (WHO, 2004, 2005). Adults are encouraged to consume as much ORS as necessary during the maintenance phase, up to approximately 2 L/day, which would provide up to 100 g/day of HAMS under its intended uses in ORS. Furthermore, exposures to HAMS from

its intended uses in ORS will only be short-term, since acute diarrhea typically lasts less than 7 days, and not longer than 14 days (Guarino *et al.*, 2008).

The recommended intakes of ORS are intended to serve as guidelines of the amount of ORS that are typically consumed to manage dehydration during acute diarrhea; in practice, individuals are encouraged to consume as much ORS as desired. As such, it is possible that the amounts of ORS consumed could deviate from these recommended values, depending on the amount of fluids lost and on the hydration status. Nevertheless, it is important to note that the recommendations on the volumes of ORS to be consumed were developed for individuals in underdeveloped nations suffering from diarrhea of all etiology, including cholera diarrhea which is associated with the greatest amount of fluid and electrolytes lost compared to diarrhea caused by other infectious agents (CDC, 2003; WHO, 2004, 2005; WGO, 2012). The amount of ORS that will be consumed by otherwise generally healthy children (>2 years) and adults in the U.S., who are unlikely to have cholera diarrhea, is likely to be less than those recommended by the authoritative bodies. For example, Vesikari *et al*. (1987) evaluated the use of rapid rehydration therapy in acute diarrhea in 37 Finnish children under the age of 5 hospitalized for acute diarrhea and dehydration. Subjects were provided oral or intravenous rehydration during 6 to 12 hours, and total fluid intake at 6 hours among ORS users during the trial ranged from 152 to 368 mL (95% CI). Dietary consumption of HAMS from this level of ORS use would equate to only 7.6 to 18.4 g HAMS per child.

6.0 INFORMATION TO ESTABLISH THE SAFETY OF HAMS IN ORAL REHYDRATION SOLUTIONS

6.1 Introduction

Given that HAMS is a natural agricultural macronutrient with an established long-history of safe consumption in the diet, toxicological evaluations in animals administered HAMS have not been conducted. Rather, the safety of HAMS as a food ingredient can be supported mainly by the fact that native food starches are generally viewed as innocuous substances that serve as a dietary carbohydrate source. There are some naturally-occurring starches that are resistant to digestion in the upper gastrointestinal tract (*i.e.*, resistant starches). Starches that are resistant to digestion in the gastrointestinal tract, such as HAMS, have a long history of safe use in the food supply, being used in a variety of food applications.

Standard glucose-based ORS help increase fluid uptake mainly by enhancing sodium-glucose mediated co-transport, which enhances net fluid absorption as a result of the osmotic gradient generated (CDC, 2003). Given that glucose serves a pivotal role in the fluid retention capacity of ORS, from a safety perspective, it will need to be demonstrated that ORS containing HAMS as the carbohydrate source are at least as effective as standard glucose-based ORS

formulations in reducing the dehydration associated with diarrhea, and does not further exacerbate the diarrhea observed. The totality of evidence to support the safety of HAMS as an ingredient in ORS intended for use by children and adults with acute diarrhea (*i.e.,* <7 days in duration), with or without signs of mild to moderate dehydration, is presented in Section 6.2 to 6.6 below.

6.2 Metabolic Fate

6.2.1 Digestibility

The digestion of starch is dependent on the action of α-amylases; although the salivary gland produces α-amylases, the primary mechanism for starch digestion in adults is mediated *via* the action of pancreatic α-amylase in the small intestines (Filer, 1988; de Sales *et al.*, 2012). The α-amylases catalyze the initial hydrolysis of the α-D-(1,4)-glycosidic bonds in starch to release shorter oligosaccharides, which are then further degraded by the action of membrane-bound α-glucosidases and other amylolytic enzymes into free glucose that are then subsequently absorbed (Topping and Clifton, 2001; de Sales *et al.*, 2012). In young infants, pancreatic amylase activity is relatively low, since enzyme levels do not reach those of adults until nearly 2 years of age (McClean and Weaver, 1993).

It has been demonstrated that the digestion of HAMS is incomplete following *in vitro* incubation with α-amylase, with approximately 30 to 55% undergoing degradation (Sandstedt *et al.*, 1962; Fujita *et al.*, 1989; Liu *et al.*, 1997). The ability of HAMS to resist digestion by amylolytic enzymes *in vivo* was first demonstrated using a rat model in 1962 (Borchers, 1962). Based on the fecal recovery of starch, it was estimated that approximately 95% of regular corn starch was digested, whereas only 66 to 71% of HAMS (amylose content of 55 to 70%) was digested in rats administered starches in the diet (Borchers, 1962). Similar results have been reported by other authors. Ikai *et al.* (1997) demonstrated that in ileorectostomized rats, the digestibility of HAMS ranged from 67 to 74%, whereas more than 99% of the regular corn starch ingested was digested. Among normal rats, the digestibility of HAMS ranged from 83 to 99%, with digestibility decreasing with increased levels of HAMS consumed in the diet (ranging from 10 to 65.5% w/w) (Ikai *et al.*, 1997).

Studies conducted using pigs and dogs have also demonstrated that HAMS is incompletely digested in the upper gastrointestinal tract, with a considerable proportion of an ingested dose entering the colon (Ackerson, 1961; Granfeldt *et al.*, 1993; Lajvardi *et al.*, 1993; Ito *et al.*, 1999; Ferguson *et al.*, 2000; Gadja *et al.*, 2005; Bird *et al.*, 2007). The digestion of HAMS in pigs is similar to that reported for humans (Topping *et al.*, 1997; Bird *et al.*, 2007). In pigs administered HAMS (85% amylose) at high dietary levels (51.5% w/w) have reported an ileal digestibility of 87.8% (Bird *et al.*, 2007). The starch content of the ileum of these pigs was double that

measured in the control animals consuming low-amylose cornstarch, and levels in the distal colon were increased to 15.4 g in animals consuming HAMS relative to controls levels of 2.6 g.

Studies conducted in humans have also demonstrated that the digestion of HAMS in the small intestines to be incomplete, with the resistant starch fraction expecting to reach the large intestines where it is fermented by the resident microflora (reviewed in Nugent, 2005; Murphy *et al.*, 2008). For example, findings from ileostomy subjects (n=7) provided 20 g of HAMS administered in cooked and cooled custards suggest the ileal digestibility of HAMS is approximately 70% (Clarke *et al*., 2007). The digestibility of uncooked HAMS in humans is likely marginally higher and closer to that observed in controlled studies in pigs where provision of HAMS or thermally treated HAMS in the diets at 51.5% (w/w) resulted in ~12 and 30% of these respective starches escaping ileal digestion (Bird *et al*., 2007).

6.2.2 Fermentation by Colonic Microflora

A large and taxonomically diverse population of bacteria resides within the large intestines, utilizing undigested dietary carbohydrates, proteins, and other nutrients, and endogenous secretions for energy (Topping *et al.*, 2008; Slavin, 2013). For example, it has been demonstrated that *Bifidobacterium* and *Clostridium* spp. exhibit efficient amylolytic activities towards high-amylose maize starch granules (Wang *et al.*, 1999; Martinez *et al*., 2010). Therefore, starches that escape digestion in the upper gastrointestinal tract are expected to undergo fermentation by the colonic microflora to release gases (H_2, CH_4, and CO_2) and short-chain fatty acids (mainly acetate, propionate, and butyrate) (Brown, 1994; Topping and Clifton, 2001). It has been well established that ingestion of all resistant starches, including HAMS, will increase the level of total short-chain fatty acids (SCFA) in the lower gastrointestinal tract and decrease cecal pH when compared to the ingestion of digestible starches (*e.g.*, regular cornstarch) in animal models (De Schrijver *et al.*, 1999; Kasaoka *et al.*, 1999a,b; Ferguson *et al.*, 2000; Saito *et al.*, 2001; Topping and Clifton, 2001; Le Leu *et al.*, 2003; Bird *et al.*, 2007). The fermentation effect of HAMS has also been investigated in a number of clinical studies, with some studies reporting that HAMS supplementation significantly increases fecal weight, decreases fecal pH, and increases fecal butyrate concentrations (reviewed in Nugent, 2005; Murphy *et al.*, 2008).

The fermentation of resistant starches is expected to be generally complete, at least among healthy individuals at levels of intake that are typically consumed in the diet (FAO/WHO, 1998; Bird *et al.*, 2010). Fermentation is normally most active in the cecum and proximal colon (Topping and Clifton, 2001), and accordingly, total SCFA concentrations are highest in the proximal colon, with levels declining as they are transported to distal regions by the fecal stream (Topping and Clifton, 2001). The molar ratio of SCFA produced from normal microbial fermentation in human colon has been reported 60:20:20 for acetate, butyrate, and propionate, respectively (Havenaar, 2011). Short chain fatty acids produced during fermentation in the

colon are rapidly absorbed by high capacity SCFA transporters, with approximately 95 to 99% of the SCFA produced being absorbed (Scheppach, 1994; Topping and Clifton, 2001). As such, SCFA concentrations in feces are not a good indicator of colonic production. The absorption of SCFA can occur through non-ionic diffusion, and by transporter-mediated mechanisms where SCFA absorption is coupled with sodium absorption (Scheppach, 1994; Hijova and Chmelarova, 2007; Binder, 2010) (see also Section 6.4).

There is no generally accepted value for concentrations of SCFA in the colon that are deemed to be normal/physiological; however, SCFA concentrations of 70 to 130 mM are expected to be present (Havenaar, 2011). Total SCFA concentrations of between 60 to 160 mM have been reported for pigs, with concentrations of acetate ranging from 40 to 120 mM, butyrate ranging from 4 to 12 mM, and propionate ranging from 10 to 50 mM are observed in the colon with levels varying according to colonic site (*i.e*., cecum *vs*. proximal, mid, or distal colon) and animal diet (Topping and Clifton, 2001; Bird *et al.*, 2007). Holtug *et al.* (1992) estimated that humans have the capacity to absorb 550 to 1,150 mmol of SCFA per day, based on the amount of SCFA that could theoretically be produced from microbial fermentation following the ingestion of 160 g/day of lactulose, subtracting the amount that was excreted as SCFA or carbohydrate.

The SCFA that are absorbed from the colon can be utilized by the viscera as an energy source, contributing 7 to 8% of the host daily energy requirements (Topping *et al.*, 2008; Slavin, 2013). It has been estimated that the energy yield from the anaerobic fermentation of dietary fibers by the colonic microflora in humans is in the range of 1.5 to 2.5 kcal/g, which is less than the energy yield from carbohydrate of 4 kcal/g (IOM, 2005). Following absorption, SCFA (particularly butyrate) can be utilized by the colonocytes as an energy source, with approximately 60 to 70% of the energy requirements of these cells being derived from SCFA oxidation (Hijova and Chmelarova, 2007; den Besten *et al.*, 2013). The liver also uses SCFA that are transported *via* portal blood as an energy source, and SCFA may serve as substrates for the synthesis of other endogenous compounds (Hijova and Chmelarova, 2007; den Besten *et al.*, 2013). Up to 70% of the acetate produced from fermentation is taken up by the liver where it is used as an energy source, as well as serving as a substrate for the synthesis of cholesterol, long-chain fatty acids, and as co-substrates for glutamine and glutamate synthesis, while propionate is a precursor for gluconeogenesis in the liver (Hijova and Chmelarova, 2007; den Besten *et al.*, 2013). Peripheral tissues (*e.g.*, heart, adipose tissue, kidney, and muscle) can also metabolize the remainder of the SCFA, particularly acetate (Hijova and Chmelarova, 2007; den Besten *et al.*, 2013). Since a large portion of the absorbed SCFA are utilized for energy by the host, changes in dietary fiber intake are not expected to significantly affect circulating levels of SCFA. For example, studies in young male Large White pigs administered HAMS at high levels (~50% w/w) produce several fold increases in the luminal concentrations of acetate, butyrate and propionate of the colon relative to levels observed in pigs consuming low amylose cornstarch diets (Topping *et al.*, 1997; Bird *et al.*, 2007); however, concentrations of

acetate, butyrate, or propionate in the portal circulation of pigs consuming the HAMS diets are not increased above those observed in the control groups (Topping *et al.*, 1997).

6.3 Toxicological Evaluation for HAMS

6.3.1 Repeated-Dose Studies

A large number of repeated dose studies have also been conducted where HAMS were administered in animal models, as summarized in Table 6.3.2-1. Various short-term studies (≤4 weeks) have been conducted where HAMS was administered at doses up to 40% in the diet [40 g/kg body weight/day (U.S. FDA, 1993)] of rats, and up to 51.5% in the diet [20.6 g/kg body weight/day (FAO/WHO, 2009)] of pigs. Overall, HAMS was well-tolerated; high dietary intakes (≥35% in the diet) may affect feed intake and impair growth in some studies (Ferguson *et al.*, 2000; Bird *et al.*, 2007), though these effects are likely attributed to caloric dilution of the diet rather than represent overt toxic effects of the ingredient. Moreover, increased cecal contents and cecal enlargement was observed in most of the studies, though such effects are considered to be a physiological response to the ingestion of large amounts of incompletely digested carbohydrates that undergo microbial fermentation in the cecum and colon (Newberne *et al.*, 1988). One study investigated the effect of HAMS on glycemic control in pregnant rats with type 2 diabetes (Shen *et al.*, 2011). Diets containing 30% HAMS [30 g/kg body weight/day (U.S. FDA, 1993)] or a control diet were administered 70 days prior to mating and throughout pregnancy and lactation, and a subset of the pups were raised to 8 weeks old on a standard diet. Litter size, body weight, body composition, growth rate, or food intake were not significantly affected in the offspring of dams fed diets containing HAMS, in comparison to the offspring of dams fed a control diet (Shen *et al.*, 2011). Although these studies were not designed for toxicological evaluation *per se*, the general lack of adverse effects provides support that the ingestion of HAMS would not pose any safety concerns.

Table 6.3.1-1 Repeated-Dose Feeding Studies Conducted with High-Amylose Maize Starch (HAMS)					
Species (Strain), Sex, Number of Animals	**Primary Study Objective**	**Test Article Administered, Dose and Duration[a]**	**Tested Parameters**	**Results Related to Safety[b,c]**	**Reference**
Rats					
Male Wistar rats, n=24	To investigate differences in the physiological effects of HAMS and HMT-HAMS on the rat cecal microflora and fermentation parameters	**Interventions**: • Control: diets containing ordinary corn starch (21% of starch content is amylose) • HAMS: provided in diet at 10% (10 g/kg bw/d) (63% of starch content is amylose) **Duration**: 10 to 13 days	Growth and diets	• NSD in final body weights • NSD in body weight gain • NSD in food intake	Saito *et al.*, 2001
			Cecal variables	• NSD in cecal microflora composition • SS ↓ in β-glucuronidase activity of cecal bacteria • SS ↑ in cecal SCFA (acetate, propionate, n-butyrate, iso-butyrate, and total) • SS ↓ in cecal pH	
			Fecal variables	• NSD in fecal weight • SS ↑ in fecal moisture	
			Organ contents weight	• NSD in stomach or small intestinal contents weight • SS ↑ in cecal and colorectal contents weight	
			Organ starch content	• SS ↑ in starch content in the upper/lower small intestine, cecum, feces, and daily starch output	
Male Wistar rats, n=24	To determine the physiological effects of retrograded amylose prepared from HAMS in rats	**Interventions**: • Control: diets containing sucrose as the CHO source (~69%) • Retrograded HAMS: Hylon VII® in the diet (~10%) (10 g/kg bw/d) **Duration**: 14 days	Growth and diets	• NSD in final body weights • NSD in food intake	Gee *et al.*, 1991
			Ileal and cecal variables	• SS ↑ in ileal wet and dry weight • SS ↑ in ileal CHO content • SS ↑ in cecal wet and dry weight • SS ↑ in cecal CHO content • SS ↓ in cecal pH	
			Fecal variables	• SS ↑ in fecal dry weight • SS ↑ in fecal CHO content • SS ↑ in fecal moisture content	
			Small intestine	• NSD in small intestine length	

Table 6.3.1-1 Repeated-Dose Feeding Studies Conducted with High-Amylose Maize Starch (HAMS)

Species (Strain), Sex, Number of Animals	Primary Study Objective	Test Article Administered, Dose and Duration[a]	Tested Parameters	Results Related to Safety[b,c]	Reference
			morphology & cell proliferation	• SS ↓ in villous length/width and crypt length • SS ↑ in ileal crypt cell proliferation rate • NSD in jejuna crypt cell proliferation rate • NSD in protein content of the jejuna mucosa • SS ↓ in sucrose and alkaline phosphatase activities	
			Serum chemistry	• NSD in serum cholesterol, plasma glucagon, and plasma enteroglucagon levels	
Male Sprague-Dawley rats, n NR	To examine the effects of HAMS on cecal and fecal variables	**Interventions**: • Control: diets containing 60% corn starch and 5.5% sucrose • HAMS: Hi-Maize® (83% amylose) in diet at 10% (10 g/kg bw/d) • HAMS: Hi-Maize® (83% amylose) in diet at 20% (20 g/kg bw/d) • HAMS: Hi-Maize® (83% amylose) in diet at 40% (40 g/kg bw/d) **Duration**: 4 weeks	Growth and diets	• NSD in body weight gain • NSD in food intake	Kasaoka *et al.*, 1999a
			Cecal variables	• NSD in GI transit time • SS ↑ in cecal tissue weight [≥10%] • SS ↑ in cecal contents weight [≥10%]	
			Fecal variables	• SS ↑ in fecal number, wet/dry weight, and moisture [≥20%] (except in week 1 for fecal number for the 10% diet group) • SS ↑ in fecal volume [≥10%]	
Male Sprague-Dawley rats, n NR	To examine the effects of HAMS on cecal and fecal variables	**Interventions:** • Control: diets containing corn starch (65.5% as CHO source) • HAMS: Hi-Maize® (83% amylose) in diet at 2.5% (2.5 g/kg bw/d) • HAMS: Hi-Maize® (83% amylose) in diet at 5% (5 g/kg bw/d) • HAMS: Hi-Maize® (83% amylose) in diet at 10% (10 g/kg bw/d) • HAMS: Hi-Maize® (83% amylose) in diet at 20% (20 g/kg bw/d)	Growth and diets	• NSD in body weight gain • NSD in food intake	
			Cecal variables	• SS ↑ in cecal tissue weight [≥5%] • SS ↑ in cecal contents weight [≥5%] • SS ↑ propionate in cecal contents [20%] • SS ↑ cecal SCFA propionate [≥5%], acetate, n-butyrate, succinate [≥10%] • SS ↑ in cecal ammonia [≥10%] • SS ↓ cecal pH [≥10%]	
			Fecal variables	• SS ↑ fecal dry matter [20%]	

Table 6.3.1-1 Repeated-Dose Feeding Studies Conducted with High-Amylose Maize Starch (HAMS)					
Species (Strain), Sex, Number of Animals	**Primary Study Objective**	**Test Article Administered, Dose and Duration[a]**	**Tested Parameters**	**Results Related to Safety[b,c]**	**Reference**
		Duration: 16 days		• SS ↑ fecal starch [≥10%]	
Male Sprague-Dawley rats, n NR	To examine the effects of HAMS on cecal and fecal variables	**Interventions:** • Control: diets containing corn starch (65.5% as CHO source) • HAMS: Hi-Maize® (83% amylose) in diet at 20% (20 g/kg bw/d) **Duration:** 10 days	Growth and diets	• NSD in body weight gain • SS ↓ in food intake	
			Cecal variables	• SS ↑ cecal tissue and contents weight	
			Fecal variables	• SS ↑ fecal dry matter, starch, nitrogen	
Male Sprague-Dawley rats, n NR	To examine the effects of HAMS on fecal variables and SCFA levels	**Interventions:** • Control: diets containing corn starch (65.5%) • HAMS: Hi-Maize® (83% amylose) in diet at 2.5% (2.5 g/kg bw/d) **Duration**: 4 weeks	Growth and diets	• NSD in body weight gain • NSD in food intake	Kasaoka *et al.*, 1999b
			Cecal variables	• SS ↑ cecal contents weight • SS ↓ cecal pH • SS ↑ cecal acetate, propionate, n-butyrate, and total SCFA	
			Colonic variables	• NSD in colonic variables	
			Fecal variables	• NSD in fecal wet/dry weight • NSD in fecal volume • SS ↑ fecal starch • NSD in fecal pH • SS ↑ fecal propionate and total SCFA; NSD in fecal acetate and n-butyrate	
Male Wistar rats, n = 64	To examine the effect of HAMS on intestinal fermentation, mineral absorption, and lipid metabolism	**Interventions:** • Control: diets containing wheat starch (73%) • HAMS: Hi-Maize® (61% amylose) in diet at 20% (20 g/kg bw/d) plus wheat starch (53%) **Duration**: 21 days	Growth	• NSD in final body weight	Lopez *et al.*, 2001
			Cecal variables	• SS ↑ in cecal weight • SS ↑ in cecal wall weight • SS ↓ in cecal pH due to ↑ SCFA (acetate, propionate, butyrate, and total)	
			Mineral absorption	• SS ↑ in absorption (and ↓ in excretion) of Ca, Zn, Fe, Mg, Cu	
			Lipid metabolism	• SS ↑ in plasma and liver cholesterol	

Table 6.3.1-1 Repeated-Dose Feeding Studies Conducted with High-Amylose Maize Starch (HAMS)					
Species (Strain), Sex, Number of Animals	**Primary Study Objective**	**Test Article Administered, Dose and Duration[a]**	**Tested Parameters**	**Results Related to Safety[b,c]**	**Reference**
				and TG concentrations • SS ↑ in excretion of cholesterol, bile, total neutral sterols • SS ↓ in total steroid balance	
Male Wistar rats, n=24	To examine whether the physiologic effects of HAMS are affected by gelatinization or HMT	**Interventions:** • Control: diets containing 40% gelatinized corn starch • HAMS: Nisshoku® (68% amylose), in diet at 40% (40 g/kg bw/d) **Duration:** 21 days	Growth and diets	• NSD in body weight gain • NSD in food intake	Kishida *et al.*, 2001
			Digestibility	• SS ↓ apparent starch and protein digestibilities	
			Mineral absorption	• NSD in absorptions of Ca, Fe, Mg, Zn	
			Lipid metabolism	• SS ↓ in plasma TG, cholesterol (total, HDL, LDL+VLDL, phospholipids) • SS ↓ in liver lipids • NSD in liver cholesterol • NSD in liver weight • SS ↓ in weight of epididymal fat pads (absolute and relative to body weight)	
			Cecal variables	• SS ↑ in cecal weight and area • SS ↑ in cecal contents weight • NSD in cecal moisture • SS ↓ in cecal pH • SS ↑ in cecal n-butyric and succinic SCFA; NSD in other SCFA	
			Fecal variables	• SS ↑ in fecal wet weight • SS ↑ in bile excretion • NSD in fecal moisture or neutral sterol excretion	
Male Wistar rats, n=24	To examine the effects of 3 RS preparations on GI function	**Interventions**: • Control: diets containing 35% normal corn starch (16.5% RS) • HAMS: provided in the diet at 35% (35 g/kg bw/d) (61.8% RS)	Growth and diets	• Diets were all well-tolerated • NSD in food intake • SS ↓ in body weight gain	Ferguson *et al.*, 2000
			GI tract variables	• SS ↓ in GI transit time (at 20% recovery); NSD at 50% or 80%	

Table 6.3.1-1 Repeated-Dose Feeding Studies Conducted with High-Amylose Maize Starch (HAMS)

Species (Strain), Sex, Number of Animals	Primary Study Objective	Test Article Administered, Dose and Duration[a]	Tested Parameters	Results Related to Safety[b,c]	Reference
	in rats	**Duration**: 3 weeks		recovery • SS ↑ in total starch content • SS ↑ in SCFA content	
			Cecal variables	• SS ↑ in fresh weight of cecum • SS ↓ in water in cecal contents • SS ↑ in total SCFA in cecum	
			Fecal variables	• SS ↑ in fresh weight of feces • NSD in water content of feces	
Female Goto-Kakizaki rats, n=20	To investigate the effects of dietary RS on glycemic control in a pregnant type 2 diabetic rat model	**Interventions**: • Control: diets containing 100% amylopectin corn starch • HAMS: Hi-Maize® (60% amylose, 40% amylopectin) in diet at 30% (30 g/kg bw/d) **Duration**: 70 days prior to mating, and diets were maintained throughout the pregnancy and weaning.	Growth and diets (maternal effects)	• NSD in food intake • NSD in disemboweled body weights • SS ↓ in %body fat/disemboweled body weight (total, abdominal, ovarian, and perirenal) • No or minimal discomfort with the diet intake was reported • No to minimal side effects on pregnancy	Shen *et al.*, 2011
			Hormone levels (maternal effects)	• SS ↑ in insulin sensitivity (as indicated by ↓ HOMA-IR) • SS ↓ in fasting glucose and serum insulin; NSD in ΔAUC • NSD in pancreatic insulin content • SS ↑ in β-cell relative densities • SS ↑ in serum total GLP-1	
			Cecal variables (maternal effects)	• SS ↓ in cecal pH • SS ↑ in cecal and GI contents weight • SS ↑ in cecal SCFA (acetate, butyrate, propionate) • SS ↑ in cecal bacterial populations (except Clostridial cluster XIV)	
			Fecal variables (maternal effects)	• SS ↓ in fecal pH	

Table 6.3.1-1 Repeated-Dose Feeding Studies Conducted with High-Amylose Maize Starch (HAMS)					
Species (Strain), Sex, Number of Animals	**Primary Study Objective**	**Test Article Administered, Dose and Duration[a]**	**Tested Parameters**	**Results Related to Safety[b,c]**	**Reference**
			Offspring effects (10 male pups, 8 weeks old)	• NSD in litter size • NSD in body weight, %body fat, or growth rate • NSD in food intake • SS ↓ in fasting glucose • NSD in fasting serum insulin, 2-h glucose level and insulin sensitivity (HOMA-IR) • SS ↑ in pancreatic insulin content • NSD in β-cell density • NSD in cecal pH	
Female Sprague-Dawley rats, n=68	To study the effect of various sugars and Hylon 7 (HAMS) on parameters of colon carcinogenesis in rodents	**Interventions**: • Control: diets containing 46% Globzeta corn starch (30% amylose, 70% amylopectin) • HAMS: Hylon 7 (70% amylose) in diet at 23% (23 g/kg bw/d) with 23% Globzeta corn starch **Duration**: 1 month	Colonic mucosal proliferation	• NSD in proliferative activity in the colonic mucosa • NSD in the number of cells per crypt column • NSD in the distribution of proliferative activity along colonic crypts	Caderni *et al.*, 1996
			Cecal variables	• SS ↓ in cecal pH • NSD in SCFA in wet cecal content • SS ↑ in cecal propionic and valeric SCFA; NSD in acetic, butyric, and isovaleric SCFA	
Pigs					
Male Large White pigs, n=24	To compare the effects of HAMS on the distribution of SCFA and related variables in pig colon	**Interventions**: • Control: diets containing all low-amylose corn starch • Low-HAMS: Hi-Maize® (44% of starch) in diets providing 3.4 MJ/d and 56% low-amylose corn starch • High-HAMS: in diets (94% of starch) providing 7.3 MJ/d and 6% wheat bran	Growth and diets	• NSD in final body weights • All diets were palatable	Topping *et al.*, 1997
			Plasma lipids	• NSD in volume of gall bladder bile • SS ↓ lithocholate and deoxycholate bile acids • NSD in biliary cholesterol and total bile acids	
			Plasma SCFA	• SS ↓ in total portal venous SCFA, acetate, and butyrate [high-HAMS];	

Table 6.3.1-1 Repeated-Dose Feeding Studies Conducted with High-Amylose Maize Starch (HAMS)

Species (Strain), Sex, Number of Animals	Primary Study Objective	Test Article Administered, Dose and Duration[a]	Tested Parameters	Results Related to Safety[b,c]	Reference
		Duration: 3 weeks		NSD in propionate	
			Fecal variables	• SS ↑ large bowel length [high-HAMS] • NSD in colon length • NSD in mass of total digesta • NSD in total and individual SCFA • NSD in starch concentration • SS ↑ fecal propionate [high-HAMS]; NSD in fecal acetate, butyrate, and total SCFA	
Large White crossbred pigs (13 male, 11 female), n=24	To compare HAMS and HMT-HAMS on large bowel fermentation effects and microbiology in pigs	**Interventions**: • Control: diets containing Mazaca 3401C low-amylose corn starch • HAMS: (85% amylose) in diets at 51.5% (51.5 g/kg bw/d) **Duration**: 21 days	Growth and diets	• NSD in final body weight • SS ↓ in body weight gain • NSD in feed conversion efficiency • All diets were well-accepted	Bird *et al.*, 2007
			Starch digestibilities	• SS ↑ in fecal starch excretion • NSD in starch levels and digestibilities in the ileal or distal colonic digesta	
			Cecal variables	• SS ↑ in cecal weight • SS ↑ in cecal wet digesta weight • SS ↓ in cecal moisture content • SS ↓ in cecal, colonic, and fecal pH • SS ↑ in cecal propionate and total SCFA; NSD in acetate and butyrate	
			Colonic variables	• SS ↑ in colon weight and length • SS ↑ in colonic wet digesta weight • SS ↓ in proximal and distal colonic moisture content • SS ↓ in colonic pH • SS ↑ in fecal acetate, butyrate, propionate, and total SCFA • SS ↑ in total anaerobic bacteria and lactobacilli in the distal colonic digesta	
			Fecal variables	• SS ↓ in fecal pH	

Table 6.3.1-1 Repeated-Dose Feeding Studies Conducted with High-Amylose Maize Starch (HAMS)					
Species (Strain), Sex, Number of Animals	**Primary Study Objective**	**Test Article Administered, Dose and Duration[a]**	**Tested Parameters**	**Results Related to Safety[b,c]**	**Reference**
				• SS ↑ in fecal lactobacilli and bifidobacteria; NSD in other microbes	

↓ = decrease; ↑ = increase; AUC = area under the curve; bw = body weight; Ca = calcium; CHO = carbohydrate; Cu = copper; d = day; Fe = iron; GI = gastrointestinal; GLP = glucagon-like peptide; HAMS = high-amylose maize starch; HDL = high-density lipoprotein; HOMA-IR = homeostatic model assessment – insulin resistance; HMT = heat-moisture treated; LDL = low-density lipoprotein; Mg = magnesium; n = number of animals; NR = not reported; NSD = no statistical differences; RS = resistant starch; SCFA = short-chain fatty acids; SS = statistically significant; TG = triglycerides; VLDL = very low-density lipoprotein; *vs.* = *versus*; Zn = zinc.

[a] Dietary concentrations of test articles (%) were converted to g/kg bw/d using the conversion factors provided by U.S. FDA (1993).

[b] Only results related to the HAMS intervention group are presented. The study may have assessed the effects of other test articles (*e.g.*, raw potato starch), but the outcomes from these interventions are not discussed in this table.

[c] Statistical comparisons were made between the HAMS *vs*. control group, unless otherwise stated. If there was more than one HAMS comparison group, results in square brackets [ ] correspond to the doses at which the effects were observed.

6.3.2 Tolerability of HAMS in Humans

In general, the ingestion of incompletely-digested, fermentable carbohydrates, particularly at high doses, is associated with undesirable gastrointestinal-related side effects such as abdominal discomforts, bloating, and flatulence (Livesey, 2001; Grabitske and Slavin, 2009). Low-digestible carbohydrates could also have laxative effects since the fraction that escapes fermentation will be excreted, thereby increasing the bulk and water content of stools (Grabitske and Slavin, 2008). A number of factors can influence the effect of low-digestible carbohydrates on gastrointestinal function and their tolerability; these include the chemical nature of the low-digestible carbohydrate, conditions under which they are consumed (*e.g.*, food matrix, divided doses compared to 1 large bolus dose), and characteristics of the individual (Livesey, 2001; Grabitske and Slavin, 2008). In turn, these factors can influence gastrointestinal motility and transit time, enzyme activity, and the intestinal microflora, which can all affect how low-digestible carbohydrates are digested, absorbed and excreted (Grabitske and Slavin, 2008).

A large number of clinical studies has been conducted to evaluate the various health effects of HAMS (*e.g.*, intestinal/colon health and glycemic response) specifically in human subjects (Table 6.3.3-1). Overall, intake of HAMS was well-tolerated when administered to generally healthy subjects. Some commonly reported gastrointestinal effects included mild laxation (*e.g*., increased ease of defecation and increased stool frequency), significant increase in scores of gastrointestinal symptoms (*e.g.,* flatulence, bloating) at doses of 20 g/day or higher (van Munster *et al*., 1994a; Muir *et al*., 1995b, 2004; Phillips *et al*., 1995; Heijnen *et al*., 1996, 1998). Moreover, in one dose-response study to specifically address gastrointestinal tolerability, administration of up to 60 g/day of resistant starch did not result in significantly differences in bowel frequency, bloating, abdominal pain, flatulence, or gastrointestinal upset compared to placebo control (Kendall *et al.*, 2003). In a systematic review of the literature pertaining to the gastrointestinal side effects of low-digestible starch, Grabitske and Slavin (2009) estimated the acceptable daily intakes of resistant starches (RS_2 and RS_3) from various sources to be approximately 45 g/day, based on the increased incidence of excessive flatulence reported at higher doses. It was also noted that resistant starches have a high laxation threshold compared to other non-glycemic carbohydrates, with only rare cases of diarrhea reported even at doses as high as 80 g/day (Grabitske and Slavin, 2009).

Given that ORS containing HAMS will be administered to individuals already with acute diarrhea, gastrointestinal tolerability is not as much of a safety concern as any potential impediments on hydration or further worsening of the diarrhea. However, as discussed in Section 6.4, the inclusion of HAMS as a replacement for glucose in ORS is not expected to result in adverse effects in subjects with acute diarrhea.

Table 6.3.3-1 Gastrointestinal Tolerability of High-Amylose Maize Starch (HAMS) from Clinical Studies						
Study Design	**Main Objective**	**Study Population[a]**	**Test Article & Dose**	**Duration**	**Results Relevant to Safety[b,c]**	**Reference**
Randomized, cross-over (blinding NR)	To test the effect of RS on fecal bulk and fermentation-dependent effects	11 healthy men and women (BMI ~18 to 32 kg/m^2) Age 22 to 54 years	High-RS diet with corn bread prepared from Hi-Maize® kernels (85% amylose)[d]; RS content 26 to 50 g/d Low-RS diet (control) with corn bread prepared from low-amylose maize kernels (0% amylose)[e]; RS content 3 to 8 g/d	3-week intervention, washout NR Preceded by a 2-week run-in period	• NSD in body weight changes • ↑ Flatulence and ease of defecation in high-RS diet *vs.* low-RS diet; NSD in other GI symptoms or diarrhea • ↑ Fecal output • ↑ Fecal starch and NSP levels • ↓ Fecal pH • ↑ Fecal SCFA levels (acetate, butyrate, total) • NSD in fecal lactate	Phillips *et al.*, 1995
Randomized, cross-over, double-blind	To test the effect of HAMS on insulin sensitivity in overweight adults	33 overweight but generally healthy men and women (waist circumference ≥89 cm for women and ≥102 cm for men) Age 18 to 69 years	HAMS (Hi-Maize® 260, containing ~60% RS) Control provided similar amount of digestible starch Dose: 0, 15, or 30 g/day of RS	4-week intervention with 3-week washout	• NSD in body weight, waist circumference, or blood lipids • NSD in frequencies of AE • NSD in score on GI tolerability questionnaire (gas/bloating, nausea, loose stools, constipation, GI cramping) • ↑ Insulin sensitivity in men for all doses; NSD in women • ↑ Acetate levels in women [30 g/d]; NSD for all other SCFA levels • NSD in all other laboratory values	Maki *et al.*, 2012
Randomized, single-blind, Latin-square design	To investigate the effects of RS on serum total CH and TG levels in healthy, normolipidemic subjects	56 healthy men and women (BMI ~22.3 kg/m^2) Age 18 to 69 years	HAMS (Hylon VII, containing ~55% RS) Control (glucose) supplemented diet had otherwise identical nutrient composition Dose: 0 or 30 g/d of RS	3-week intervention, washout NR	• No dropouts related to test article • NSD in body weight change • No treatment effects were observed for fasting serum total CH, HDL and LDL-CH, TG, and 3α-hydroxy bile acids after 3 weeks of intervention • ↑ Flatulence and bellyache • NSD in number of bowel movements per day • Softer stools	Heijnen *et al.*, 1996

Table 6.3.3-1 Gastrointestinal Tolerability of High-Amylose Maize Starch (HAMS) from Clinical Studies						
Study Design	**Main Objective**	**Study Population[a]**	**Test Article & Dose**	**Duration**	**Results Relevant to Safety[b,c]**	**Reference**
Randomized, single-blind, balanced multiple cross-over, orthogonal Latin-square design	To investigate whether addition of RS in diets would affect risk factors for colon cancer in healthy men	24 healthy men (BMI 22.7 ± 1.8 kg/m^2) Age 23 ± 2 years	HAMS (Hylon VII, containing ~63% RS) Control (glucose) supplemented diet had otherwise identical nutrient composition Dose: 0, or 32 g/d of RS	1-week intervention, washout NR Preceded by a 1-week run-in period	• No dropouts were reported • NSD in body weight change • ↑ Fecal number and weight • NSD in fecal consistency • NSD in fecal wet/dry weight and pH • NSD in pH, cytotoxicity, and osmolarity of fecal water • NSD in total and individual concentrations of fecal SCFA • NSD in concentration of bile acids in fecal water • ↑ Flatulence and bloating; NSD in other GI symptoms • No severe side effects	Heijnen *et al.*, 1998
Randomized, cross-over (blinding NR)	To assess the metabolic effects of specific starches on glucose, insulin, lipid profiles, and bowel function	23 overweight men and women with abdominal obesity (BMI >25 g/m^2; waist-hip ratio >0.9 for men and >0.8 for women), high plasma TG concentrations (>2.0 mmol/L), or mild hypertension (>140/90 mg Hg) Age 44 to 64 years	Hi-amylose diet with HAMS (Hi-Maize®, containing 85% amylose and ~33% RS), providing 28 to 37 g of total dietary fiber Low-amylose diet (control) providing 11 to 13 g of total dietary fiber Both groups were provided with a background diet[f] Dose: 0 or 50 g of RS (women); 0 or 74 g of RS (men)	4-week intervention; no washout	• Test foods were “acceptable” • NSD in body weight change • NSD in plasma total CH, LDL, or HDL-CH • NSD in fasting plasma glucose/insulin; ↑ postprandial plasma glucose/insulin • ↑ Fecal frequency • NSD in fecal weight • ↓ Fecal pH • ↑ Concentration of SCFA butyrate in fecal water • NSD in total fat, lithocholic acid, acetate, and propionate excretion	Noakes *et al.*, 1996

Table 6.3.3-1 Gastrointestinal Tolerability of High-Amylose Maize Starch (HAMS) from Clinical Studies						
Study Design	**Main Objective**	**Study Population[a]**	**Test Article & Dose**	**Duration**	**Results Relevant to Safety[b,c]**	**Reference**
Randomized, cross-over (blinding NR)	To compare the effects of two RS against wheat bran in areas related to colonic function, glycemic control, and serum lipid metabolism	24 healthy men and pre-menopausal women (BMI ~19 to 34 kg/m^2) Age 22 to 53 years	HAMS (source NR, containing~44% RS) Control (wheat bran) provided similar amounts of total dietary fiber Breakfast cereals and muffins served as vehicles for the intervention groups Dose: 0 or 21.5 g/d of RS	2-week intervention with 2-week washout	• NSD in body weight changes • NSD in compliance • ↑ Fecal output • No changes in total and individual fecal SCFA • NSD in change from baseline, or end-of-treatment values for serum urea or creatinine • NSD in change from baseline, or end-of-treatment values for serum lipids and lipoproteins	Jenkins *et al.*, 1998
Randomized, cross-over, block-design (blinding NR)	To investigate whether diets containing wheat bran and RS could produce more favorable changes in fecal variables than wheat bran alone	20 healthy men and women with a family history of colorectal cancer (BMI 19 to 31 kg/m^2) Age 22 to 67 years	High-RS diet with HAMS (Hi-Maize®, 85% amylose) plus wheat bran (RS content = 20 to 30 g/d) Low-RS diet with wheat bran provided similar amounts of dietary fiber and digestible starch (RS content = 2 to 5 g/d) Both groups were provided with a background diet[g]	3-week intervention with 1-week washout	• NSD in body weights from baseline • Moderate flatulence; no other GI symptoms were reported • ↑ Fecal output (wet and dry weight) • ↑ Fecal starch • ↓ Fecal pH • NSD in fecal frequency or transit time • ↓ Fecal concentrations of phenols, p-cresol, and ammonia • ↑ Fecal SCFA concentrations (acetate, propionate, isobutyrate, butyrate, isovalerate, and total)	Muir *et al.*, 2004

Table 6.3.3-1 Gastrointestinal Tolerability of High-Amylose Maize Starch (HAMS) from Clinical Studies						
Study Design	**Main Objective**	**Study Population[a]**	**Test Article & Dose**	**Duration**	**Results Relevant to Safety[b,c]**	**Reference**
Cross-over (randomization, blinding NR)	To examine the effects of RS on fermentative activity and related aspects of colonic metabolism	12 healthy men and women (BMI ~18 to 29 kg/m^2) Age 23 to 28 years	HAMS (Hylon VII) Control (corn starch) provided similar amounts of dietary fiber and available starch Dose: 7.7 or 55.2 g/d of RS	4-week intervention with 6-week washout	• NSD in mean body mass index • Well-tolerated; NSD in GI symptoms and no diarrhea was reported by any subjects • ↑ Fecal starch excretion • ↑ Breath hydrogen concentrations • ↑ Fecal wet/dry weights • NSD in fecal water content, pH, or mean transit time • NSD in fecal concentration and excretion of SCFA • ↑ Fecal excretion of malondialdehyde • ↓ Fecal bacterial β-glucosidase activity; NSD in fecal bacterial β-glucuronidase, sulfatase, and nitroreductase activities • ↓ Fecal concentrations of total and individual neutral sterols; NSD in excretion of neutral sterols • ↓ Fecal concentrations of total bile acids; NSD in excretion of bile acids	Hylla *et al.*, 1998
Randomized, cross-over (blinding NR)	To examine the starch digestibilities of 2 meals similar in nutrient composition but differing in RS content	5 men and women that received total ileostomies for ulcerative colitis (BMI 22 to 27 kg/m^2) Age 39 to 67 years	High-RS diet with corn bread prepared from Hi-Maize® kernels (85% amylose)[d] Low-RS diet (control) with corn bread prepared from low-amylose maize kernels (0% amylose)[h] Dose: 1.6 or 18.5 g of RS	30-hour intervention with 1-week washout	• NSD in small intestinal transit time • No subjects reported GI discomfort from consuming the high-RS diet	Muir *et al.*, 1995a
Randomized,	To measure	8 healthy men and	High-RS diet with corn	28-hour	• Mild diarrhea was reported by 2	Muir *et al.*,

Table 6.3.3-1 Gastrointestinal Tolerability of High-Amylose Maize Starch (HAMS) from Clinical Studies						
Study Design	**Main Objective**	**Study Population[a]**	**Test Article & Dose**	**Duration**	**Results Relevant to Safety[b,c]**	**Reference**
cross-over (blinding NR)	the effect of 2 diets differing in RS content on colonic fermentation	women (BMI~19 to 25 kg/m^2) Age 24 to 40 years	bread prepared from Hi-Maize® kernels (85% amylose)[d] Low-RS diet (control) with corn bread prepared from low-amylose maize kernels (0% amylose)[i] Dose: 5.2 or 59.1 g of RS	intervention with ≥1-week washout	subjects during the high-RS diet • ↑ Flatulence, abdominal distension, and abdominal cramps • ↑ Breath hydrogen concentration • ↑ Serum acetate SCFA concentration	1995b
Cross-over (randomization, blinding NR)	To determine the effects of RS on colonic fermentation in healthy subjects	19 healthy men (other characterization NR) Age 21 to 76 years	HAMS (Hylon VII, containing ~62% RS) Control (maltodextrin) provided similar amounts of starch and dietary fiber Dose: 0 or 45 g/d of RS	1-week intervention with 1-week washout	• Well-tolerated; only flatulence was somewhat increased (significance NR)	van Munster *et al.*, 1994a
Non-randomized	The effect of RS on bile acid metabolism and colonic proliferation in humans	13 healthy men and women (other characterization NR) Age 28 to 73 years	HAMS (Hylon VII, containing ~62% RS) Control (maltodextrin) given during the control period provided similar amounts of starch and dietary fiber Dose: 0 or 45 g/d of RS	2-week intervention, preceded by a 1-week control period	• Body weights did not change during the course of the study • 1 subject dropped out due to abdominal cramps and discomfort after starting HAMS; other 13 subjects tolerated the HAMS well • NSD in stool consistency and bowel frequency • ↑ Fecal wet weight; NSD in dry weight • NSD in fecal pH • ↑ Fecal SCFA excretion (acetate, propionate, butyrate, and total); NSD in valerate • ↑ Fecal bile acids	van Munster *et al.*, 1994b

Table 6.3.3-1 Gastrointestinal Tolerability of High-Amylose Maize Starch (HAMS) from Clinical Studies						
Study Design	**Main Objective**	**Study Population[a]**	**Test Article & Dose**	**Duration**	**Results Relevant to Safety[b,c]**	**Reference**
					• ↓ Cytotoxicity of individual fecal samples • ↓ Colonic mucosal proliferation	
Randomized, placebo-controlled, parallel	To determine the effects of RS on biomarkers of colonic fermentation and proliferation of colonic mucosal cells	23 men and women with recently removed colonic adenomas Age 26 to 73 years	RS (Hylon VII, containing ~62% RS) Control (maltodextrin) group provided similar amounts of dietary fiber Dose: 0 or 45 g/d of RS	4-week intervention Preceded by a 4-week run-in period	• All subjects tolerated the intervention well without GI complaints • NSD in total or individual proliferating cells, fecal pH, total or individual excretion of SCFA, fecal wet/dry weights • NSD in total bile acids in feces/excretion of bile acids • ↓ total soluble bile acids in fecal water and % secondary bile acids in fecal water	Grubben *et al.*, 2001
Randomized, cross-over	To assess the effect and tolerance of RS in the diet on symptoms of fermentation	24 healthy subjects (other characterization NR) Age NR	RS (source NR) Control starch included for comparison Dose: 0, 30, 45, or 60 g of RS	1-week intervention with ≥1-week washout	• No effect on body weight or blood pressure • NSD in bowel frequency, abdominal distension, flatulence, or GI upset	Kendall *et al.*, 2003 [abstract]

AE = adverse events; BMI = body mass index; CH = cholesterol; d = day; GI = gastrointestinal; HAMS = high-amylose maize starch; HDL = high-density lipoprotein; LDL = low-density lipoprotein; NR = not reported; NSD = no significant differences; NSP = non-starch polysaccharides; RS = resistant starch; SCFA = short chain fatty acids; TG = triglycerides.

[b] Reported as number of subjects that completed the study.

[b] Only results related to the HAMS intervention group are presented. The study may have assessed the effects of other test articles (*e.g.*, retrograded HAMS), but the outcomes from these interventions are not discussed in this table.

[c] Statistical comparisons were made between the HAMS *vs*. control group, unless otherwise stated.

[d] In addition to the corn bread prepared using ground high-amylose maize kernels, the high-RS diet was also supplemented with ground unprocessed wheat seeds and raw green banana flour.

[e] In addition to the corn bread prepared using ground low-amylose maize kernels, the low-RS diet was also supplemented with biscuits made from steamed, flaked wheat seeds and cooked green banana flour. Additional NSP-rich maize bran was added to the cornbread in order to ensure equivalent NSP contents between the high- and low-RS diets.

[f] The background diets were low in fiber and fat, and consisted of muffins, bread, breakfast cereal, and pasta to which the high- or low-amylose supplements were added.

[g] Subjects were supplied with cornbread, muffins, cakes, cereals, and desserts containing high- or low-RS supplementation.

[h] In addition to the corn bread prepared using ground low-amylose maize kernels, the low-RS diet was also supplemented with biscuits made from steamed, flaked wheat seeds and cooked green banana flour. The uncooked and cooked banana and wheat flours were also incorporated into ice cream for milkshakes.
[i] In addition to the corn bread prepared using ground low-amylose maize kernels, the low-RS diet was also supplemented with biscuits made from steamed, flaked wheat seeds and cooked green banana flour. The uncooked and cooked banana and wheat flours were also incorporated into ice cream for milkshakes and to accompany desserts, or incorporated into an uncooked lemon-biscuit slice.

6.4 Water Absorption and Utility of HAMS in Oral Rehydration Solutions

6.4.1 Water Absorption in Humans

Oral rehydration therapy has been used for over 40 years in the management of the dehydration and metabolic acidosis associated with diarrheal diseases (Victora *et al.*, 2000; Binder *et al.*, 2014). Oral rehydration solutions work primarily by restoring the electrolyte balance through promotion of glucose and sodium absorption in the small intestine. A discussion of the physiological processes mediating water absorption in the gastrointestinal tract, and normal nutritive mechanisms that are targeted by oral rehydration therapy during diarrhea are presented below (Binder and Ramakrishna, 2004; Kelly and Nadeau, 2004).

Absorption of water in the small intestine and colon occurs entirely *via* passive diffusion across nanometer pores of adjacent epithelial cells in accordance with the osmotic gradient (Hall, 2011). There is no active transport of water in the gastrointestinal tract; movement of water across the gastric epithelium is driven by changes in osmotic pressure occurring from the transport of soluble molecules into and out of the intestinal lumen by active and passive transport mechanisms operating on the apical and basolateral surfaces of intestinal and colonic epithelium. Water diffuses so freely across the gut mucosa that it instantaneously "follows" transported substances to or from the circulation (Hall, 2011). Mechanistically, most cases of bacterial or viral induced diarrhea are mediated by disruption in the normal electroneutral absorption processes and electrolyte secretion (for reviews see Kopic and Geibel, 2010 and Binder *et al.*, 2014). Disruptions in the normal balance of electrolyte transport occurring as a result of intestinal infections produce high osmotic pressures within the lumen of the gastrointestinal tract, leading to rapid loss of water from the circulation. Profound diarrhea and the associated dehydration that can be fatal if not corrected by consumption of oral rehydration solutions.

The columnar epithelial monolayer is responsible for electrolyte absorption and secretion within the intestine (Figure 6.4.1-1). Electrolyte secretion occurs mainly in the crypt cells and absorption is largely restricted to the villus or surface epithelium, although some regional overlap of these processes exist (Kopic and Geibel, 2010). Electroneutral absorption of sodium is an important regular of water balance. Electroneutral absorption of sodium is a coupled transport process that involves the absorption of sodium by the Na^+, H^+-exchanger (NHE) and chloride uptake by Cl^-, HCO_3^+-exchangers. Although multiple NHE isoforms have been identified, NHE3 is believed to be the main transporter regulating electroneutral sodium uptake. The activity of NHE3 is regulated by cyclic nucleotides, including cAMP and cGMP (Kopic and Geibel, 2010).

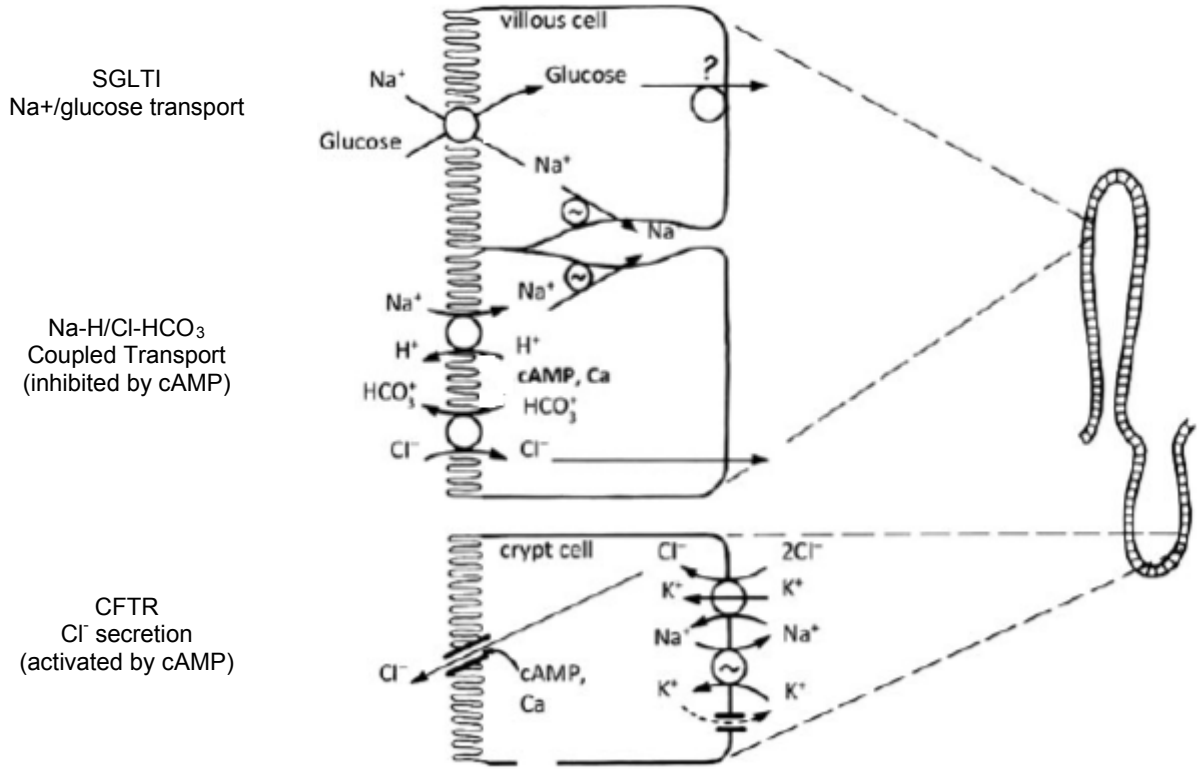
The normal metabolic processes of the gastrointestinal tract (*e.g.*, production of pancreatic juices, biliary juices and saliva) require the secretion of fluid and approximately 8 L of fluid are

secreted per day in normal healthy adults. The enterocyte cannot actively transport water into the lumen and secretory processes are therefore linked to the creation of osmotic gradients that drag water into the intestinal lumen; the apical chloride channel, cystic fibrosis transmembrane conductance regulator (CFTR), is an important driver of this osmotic force. Chloride secretion through the CFTR is regulated by cAMP, and is an active transport process that requires hydrolysis of ATP. Active transport of chloride across the apical membrane *via* the CFTR also requires a constant negative membrane potential which is generated by passive leak of potassium across the basolateral potassium channel and the coupled maintenance of intracellular chloride concentrations *via* the Na^+, K^+, $2Cl^-$ symporter (NKCC).

The absorption of water in the small intestine is also mediated by the transport of glucose *via* the sodium-glucose linked transporter (SGLT) symporter SGLT1. As 2 sodium ions are required to transport one glucose ion across the basolateral membrane, passive transport of glucose across SGLT1 creates a negative osmotic gradient within the intestinal lumen that results in significant water flow out of the chyme into the circulation.

The transport of water across the colonocyte *via* the active transport of short-chain fatty acids (derived from the activity of microbial fermentation of resistant carbohydrates entering the colon) represents another important, and perhaps, unappreciated mechanism for water absorption. The processes of SCFA and water absorption in the colon are discussed in further detail in Section 6.4.3.

Figure 6.4.1-1 Electrolyte Absorption in Small Intestine (adapted from Binder *et al.*, 2014)



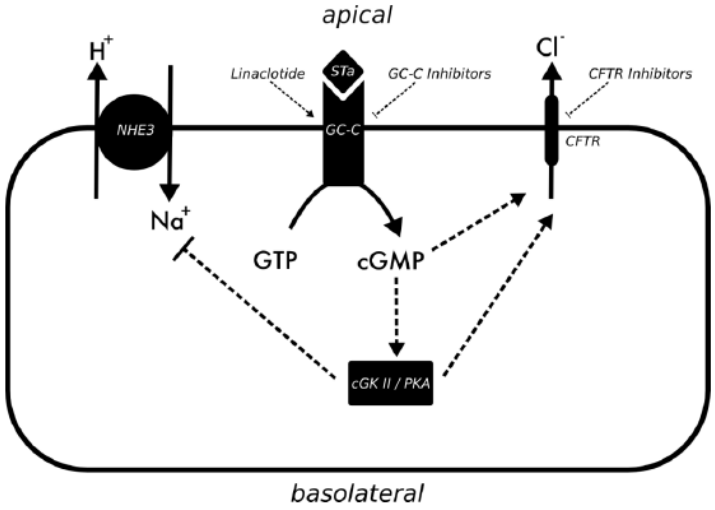
6.4.2 Water Loss during Bacterial and Viral Gastroenteritis

Enterotoxigenic *Escherichia coli* (ETEC) and *Vibrio cholera* (Vibrio cholera) are among the leading causes of diarrhea in the underdeveloped and developing world. The severe diarrhea associated these infections are attributed to the enterotoxins (*e.g.*, heat stable and heat labile enterotoxins, shiga toxin, cholera toxin) secreted by these organisms. Although the specific receptor interactions and downstream signaling events by which enterotoxins induce water loss are unique to each toxin, most toxins ultimately produce changes in intracellular signaling pathways that result in large increases in the synthesis of intracellular cyclic nucleotides (*i.e.*, cAMP, cGMP) within the enterocyte/colonocyte (for reviews see Kopic and Geibel 2010; Binder *et al*., 2014). Since cAMP/cGMP are negative regulators of the Na-H/Cl-HCO_3 co-transporter (NHE3), enterotoxins that produce increases in cAMP levels increase the osmotic pressure in the gut lumen by preventing the absorption of sodium and chloride (Figure 6.4.2-1). The osmotic pressure of the chyme is further increased by the action of enterotoxins as cAMP/cGMP activates the CFTR resulting in the secretion of large quantities of chloride into the gastrointestinal lumen. This dual effect of reduced Na^+ and Cl^- absorption and increased Cl^- secretion caused by disregulation of cAMP produce the massive water losses associated with

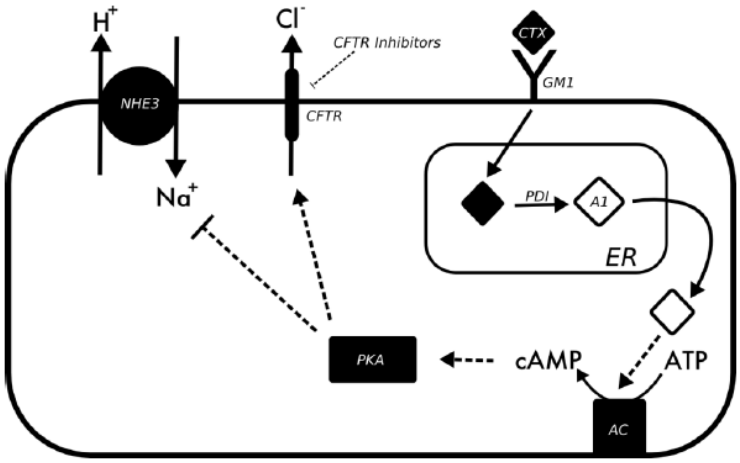
enterotoxigenic organisms. Since changes in intracellular concentrations of cyclic nucleotides do not affect the activity of the SGLT1 transporter, the fluid and electrolyte loss resulting from diarrhea can be normalized by the consumption of appropriate iso-osmolar concentrations of sodium and glucose provided within ORS. However, the utility of glucose-sodium based ORS is limited to rehydration effects since its ability to increase water absorption is limited to the small intestine. Oral rehydration solutions do not correct water loss occurring in the colon, an important mediator of water absorption, and therefore they do not appreciably lower fecal fluid losses that produce diarrhea.

Figure 6.4.2-1 Model summarizing cellular processes during (A) ETEC and (B) *Vibrio cholera* mediated diarrhea (adapted from Kopic and Geibel, 2010)

A.



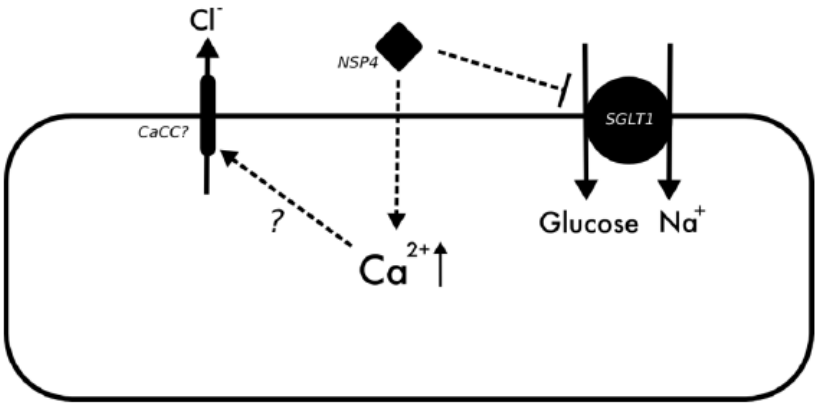
B.



Gastroenteritis from *E. coli* and other enterotoxigenic organisms such as *V. cholera*, although important causes of diarrhea in the under developed and developing nations, are rare in

developed countries. Nevertheless, diarrheal illness remains a significant problem in developed countries and in the U.S. alone 60,000 hospitalizations attributable to rotavirus related dehydration occur annually (Kopic and Geibel, 2010). The mechanism(s) of rotavirus diarrhea are poorly understood. Rotavirus RNA encodes for 6 viral proteins (VPs) and 6 nonstructural proteins (NSPs). These proteins are required for viral replication and also are responsible for various symptoms of rotavirus infection (*e.g.*, diarrhea, lactose deficiency). Experimental data provided from incubation of human intestinal cells with purified NSP4, and oral administration of NSP4 to young mice, have implicated NSP4 as a probable etiologic agent in diarrhea (Ball *et al.*, 1996; Dong *et al.*, 1997). NSP4 appears to promote intestinal water-loss in a calcium dependent manner by increasing secretion of chloride *via* transport proteins that are distinct from CFTR (Figure 6.4.2-2). There also is some experimental evidence to suggest that rotavirus infection results in the inhibition of the glucose transporter SLGT1 impairing effective absorption of Na^+ and glucose (Kopic and Geibel, 2010). Rotavirus invades the mature host epithelial cells of the upper and middle villus and can significantly impair the functioning of membrane bound disaccharidases (*i.e.*, lactase) resulting in impaired carbohydrate absorption, an effect that can be particularly problematic for infants consuming significant quantities of lactose form human milk or formula (Cotran *et al.*, 1999). Rotavirus infection also has been associated sporadic cases of hypernatremia and hypoglycemia that can complicate ORS administration in severe cases (Kaiser *et al.*, 2012). Due to the multi-factorial causes of rotavirus diarrhea, and fact that the pathogenesis of water loss is poorly understood, there is no treatment for the disease which typically resolves under proper medical care; however, the use of ORS is well established to effectively restore hydration among these individuals.

Figure 6.4.2-2 Rotavirus - Model of NSP4 mediated diarrhea (adapted from Kopic and Geibel, 2010)



6.4.3 Role of Short-Chain Fatty Acids in Colonic Absorption of Water and Utility in Oral Rehydration Solution

Since the development of the original WHO-recommended ORS, which was an iso-osmolar solution (*i.e.*, 311 mOsm/L), there has been much research efforts aimed to improve the effectiveness of ORS to promote rehydration and reduce fluid losses of diarrhea. To reduce the likelihood of hypernatremia and potential adverse effects on net fluid absorption from the high osmotic load, modified ORS formulations containing less salt and glucose (*i.e.*, hypo-osmolar ORS with 245 mOsm/L) have been developed and are endorsed by WHO/UNICEF (UNICEF, 2003; Kelly and Nadeau, 2004; Binder *et al.*, 2014). Nevertheless, one limitation of even the "new" reduced osmolarity WHO-ORS is that they do not correct water absorption in the colon, an important regulator of water balance in humans (UNICEF, 2003; Binder and Ramakrishna, 2004; Binder *et al.*, 2014). It has been proposed that it may be beneficial to use food starches as a source of glucose in ORS. As discussed, glucose is included in ORS as the co-transport of glucose and sodium into the enterocyte *via* SGLT1 is an important regulator of water absorption in the small intestine that is not inhibited by enterotoxins. Sodium and water absorption are optimal at ORS glucose concentrations of 50 mM, which produces increases in water absorption of 4- to 6-fold in the jejunum and 2- to 3-fold within the ileum (Kelly and Nadeau, 2004). Higher concentrations of glucose and sodium can produce osmotic effects that counter optimum water absorption in the intestine, a problem that was initially observed with use of standard iso-osmolar WHO ORS formulations. Hypo-osmolar ORS is now recommended (WHO/UNICEF, ESPGHAN, CDC) for effective for rehydration in adults and children with cholera and non-cholera diarrhea, including rotavirus infection (Farthing *et al.*, 2013). Despite the fact that low-osmolar ORS is recommended for all types of infection diarrhea, standard iso-osmolar ORS may reduce the incidences of hyponatremia in adults with cholera, and remains and effective rehydration option for this disease (WHO/ICDDRB, 1995). Rice-based ORS have proven to be superior to standard glucose based ORS for adults and children with cholera and is recommended when available (Farthing *et al.*, 2013). The effectiveness of rice-based ORS for cholera has been attributed to the delivery of low-osmolar glucose to the small intestine and the additive effects of delivering resistant starch to the colon where fermentation by the indigenous microflora results in the production of SCFA electrolytes that can further enhance sodium and water absorption at a site that is not affected by traditional glucose based ORS. Recent findings observed with the use of high-amylose cornstarch as an adjunct or replacement to glucose in ORS have suggested that resistant starch based ORS may prove superior to current WHO-ORS formulations for rehydration (Ramakrishna *et al.,* 2000; Raghupathy *et al.*, 2006; Ramakrishna *et al*., 2008; Alam *et al.,* 2009; Pal *et al.*, 2013). A summary of the mechanism of water absorption in the colon and role of SCFA in maintaining water balance in the colon is presented below.

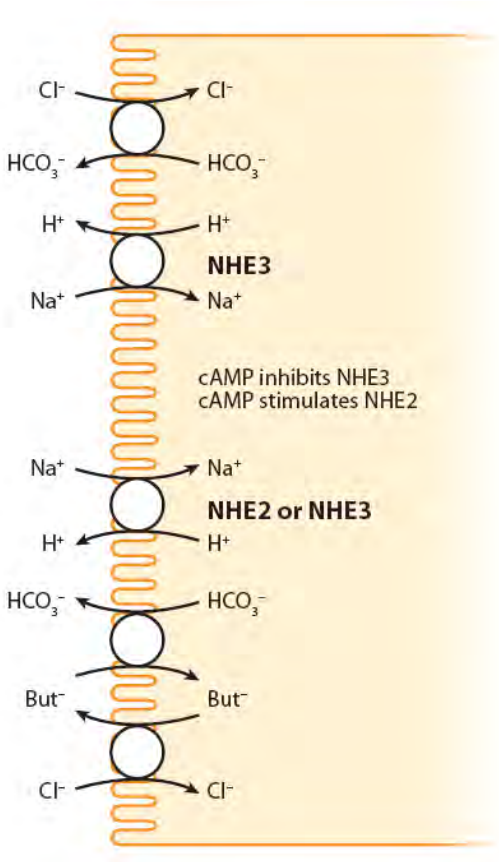
One of the primary functions of the colon is to absorb 90% of the 1.5 to 2 L of ileal effluent that is produced on a daily basis by normal digestive processes. In mammalian species, key determinants of water absorption are the rate of Na^+ and SCFA absorption (Sandle, 1998). The physiological importance of SCFA production by microbial fermentation in maintaining optimal water absorption in healthy humans is supported by the observation that antibiotic use can produce diarrhea of varying levels humans and animals. Additional evidence for the role of SCFA in water absorption is provided by studies evaluating fecal weight and breath hydrogen[4] (from microbial fermentation) in individuals provided a lactulose[5] meal with or without antibiotics. In the absence of antibiotics, lactulose consumption increases breath hydrogen and does not affect stool weight; however, during antibiotic use, consumption of lactulose produces significant increases in stool weight, daily stool frequency, and loose stools (*i.e.*, diarrhea), as well as reduced breath hydrogen levels by 44% (Rao *et al.*, 1988). Moreover, the stool water output was substantially lower among subjects who were administered lactulose compared to those administered the same amount of polyethylene glycol (PEG) (Hammer *et al.*, 1989). Both lactulose and PEG can induce osmotic diarrhea; PEG is not digested by human enzymes, is not absorbed, and is not fermented, and thus increase stool water output in a dose-dependent manner (Binder, 2010). In contrast, lactulose can be fermented to produce SCFA, which help promote fluid absorption, with diarrhea being observed only at doses that exceed the microflora's capacity to metabolize lactulose to SCFA and/or the colonic capacity for absorbing the SCFA produced (Binder, 2010). These findings suggest that water absorption in the colon is in part regulated by the normal products of microbial fermentation.

In vitro studies using isolated rat colon mucosa have suggested that significant SCFA absorption occur *via* non-ionic diffusion. It is also recognized that SCFA absorption occurs through transport mechanisms; though the specific identity of the SCFA transporters in the human colon remains unknown, experiments conducted using apical membrane vesicles from human ileum and rat distal colon have provided convincing evidence for the existence of a sodium-dependent SCFA-HCO_3 exchange system that displays saturation kinetics (reviewed in Binder, 2010). The transporter has affinity for butyrate, propionate and acetate but not lactate. Studies by Lecona *et al.* (2008) have demonstrated that two distinct butyrate transport processes exist, a low-affinity, high capacity butyrate-HCO3 exchange and a high-affinity, low capacity proton-monocarboxylate cotransporter. SLCA8, a sodium-dependent SCFA transporter with affinity for butyrate, propionate, acetate and lactate, also has been cloned from human intestine (Miyauchi *et al.*, 2004). Transport studies conducted in rat distal colon have demonstrated that active sodium and chloride absorption is enhanced by butyrate and other SCFA. Subsequent studies conducted in rats suggest the existence of a sodium dependent

[4] Fermentation of lactulose by gut microflora produce SCFA and hydrogen gas.
[5] Lactulose is a disaccharide that is not hydrolyzed by mammalian digestive enzymes, but is consumed during anaerobic fermentation in the colon. Administration of lactulose to healthy individuals therefore models the effects of lactose consumption in individuals with lactase deficiency.

butyrate-HCO_3 exchange that works in parallel with butyrate-Cl transport (Figure 6.4.3-1). This system is similar Na-H/Cl-HCO_3 coupled transport process discussed in Section 6.4.2 above that is inhibited by cAMP. However, unlike the cyclic AMP sensitive Na-H/Cl-HCO_3 exchange, the transport of butyrate through the butyrate-HCO_3 exchange is not inhibited by cAMP. *In vitro* studies using rat colonic mucosa have demonstrated that cholera toxin does not reduce butyrate stimulated sodium absorption and may in fact increase the absorption of sodium. This paradigm between cAMP mediated inhibition *vs*. stimulation effects appear to be explained by the existence of two distinct Na-H exchange isoforms (NHE2 and NHE3) involved in CHO_3-stimulated and butyrate-stimulated Na^+ absorption processes operating in the colonic apical membrane. It is interesting to speculate that the reduced incidences of hyponatremia associated with use of rice based ORS relative to standard iso-osmolar ORS may be attributed to SCFA mediated co-transport of sodium combined with the stimulatory effects of cholera toxin on the NHE2 transporter.

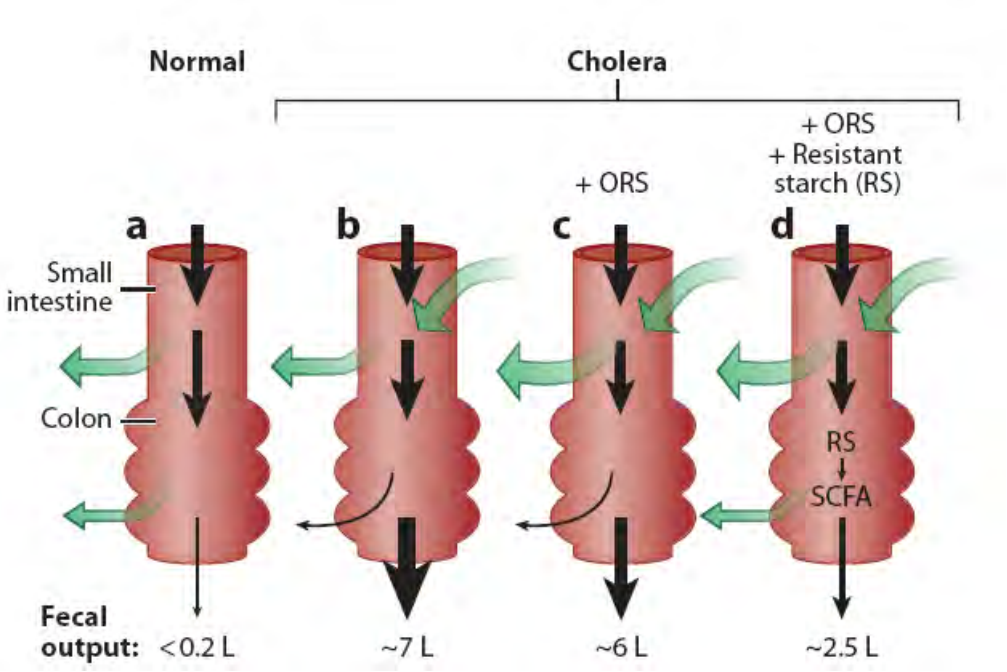
Figure 6.4.3-1 Proposed mechanism of SCFA absorption (adapted from Binder, 2010)



Current uses of ORS for treatment of dehydration of diarrhea are based on fundamental discoveries that absorptive and secretory processes are largely separate and independent, and that perturbations in Na^+ absorption and Cl^- secretion, caused by enterotoxigenic induced

elevations in the intracellular concentrations cAMP, does not affect the absorption of glucose and sodium *via* SGLT1. With the potential exception of cholera diarrhea, the use of low-osmolar glucose solutions has been determined to provide optimal absorption of electrolytes and water for most cases of bacterial and viral diarrhea. The opportunity for further improvements in ORS are provided by the use of resistant starch as an adjunct and/or replacement for glucose in ORS by providing a low-osmotic source of digestible glucose and by taking advantage of the normal role of microbial fermentation and SCFA in electrolyte and water absorption in the colon (Figure 6.4.3-2). Findings from studies in animals and humans demonstrating the effectiveness of resistant starch in treating dehydration of diarrhea are presented in Section 6.4.4 and 6.4.5 below.

Figure 6.4.3-2 Role of resistant starch in promoting rehydration and reducing fecal water output associated with enterotoxigenic diarrhea (adapted from Binder, 2010)



6.4.4 Animal Studies

Using a whole-gut perfusion model of cholera or enterotoxigenic *Escherichia coli* diarrhea in rats, Subramanya *et al.* (2006) demonstrated that replacement of glucose by HAMS in both iso-molar and hypo-osmolar ORS significantly enhanced electrolyte and water absorption in the gastrointestinal tract. In this study, either cholera toxin or the heat-stable enterotoxin of *Escherichia coli* was instilled into the small intestines of adult Wistar albino rats (number or sex not reported), which decreases the net absorption of water and electrolytes (Na^+, Cl^-, and K^+)

across the gastrointestinal tract, mimicking the effects seen in cholera and non-cholera diarrhea. The effect of various ORS differing in glucose source and osmolarity towards net fluid and electrolyte movement measured across the whole gut (*i.e.*, small intestines and the colon) was investigated. Following infusion of cholera toxin into the intestines, perfusion with ORS containing Hi-Maize® HAMS at 50 g/L (RS-ORS, 200 mmol/L) resulted in significantly high net water absorption compared with perfusion with a standard glucose ORS (311 mmol/L) or ORS containing digestible starch (DS-ORS, 200 mmol/L). Given that the effect on water absorption was greater for RS-ORS compared to DS-ORS, the effect of RS on water absorption is unlikely to be due solely to the reduced osmolarity, but is also related to some colonic component. The absorption of sodium, potassium, and chloride following perfusion with RS-ORS was also significantly higher, while the secretion of bicarbonate was significantly lower, compared to perfusion with the standard glucose-ORS. Furthermore, perfusion with a reduced osmolarity ORS (RO-ORS) containing HAMS at 50 g/L (RS-RO-ORS, 170 mmol/L) significantly increased net water absorption compared with a standard RO-ORS (245 mmol/L) or RO-ORS containing 50 g/L of rice flour (170 mmol/L). Similar results were observed in animals that were instilled the heat-stable enterotoxin of *E. coli*. Perfusion of RS-RO-ORS (170 mmol/L) significantly increased water absorption compared to the RO-ORS (245 mmol/L) or standard ORS (311 mmol/L).

Overall, the authors proposed that the ORS containing HAMS, which comprise both a digestible (~70%) and amylase-resistant fraction (~30%), may confer advantages over the standard glucose-based ORS. The advantages of using HAMS in ORS is 2-folds; HAMS can be hydrolyzed and stimulate sodium-glucose co-transport within the small intestines, without the osmotic penalty of glucose, while the amylase-resistant fraction can provide additional stimulation of sodium and water absorption within the colon.

6.4.5 Human Studies

A total of 5 studies were identified where ORS containing HAMS were administered to human subjects with diarrhea and diagnosed dehydration (Ramakrishna *et al.,* 2000, 2008; Raghupathy *et al.*, 2006; Alam *et al.,* 2009; Pal *et al.*, 2013). The characteristics of these studies are summarized in Table 6.4.5-1.

6.4.5.1 Adults with Cholera and Non-Cholera Diarrhea

Ramakrishna and colleagues (2000) conducted a randomized controlled study in 48 adult and adolescent patients (14 to 58 years) presenting to Christian Medical College and Hospital (Vellore, India) with acute, watery diarrheal illness of <72 hours caused by cholera. Subjects with confirmed Vibrio cholera infection were randomized to receive 1 of 3 therapies: 1) Standard iso-osmolar glucose based ORS (n=16; $\bar{x}$ = 36.4 yrs; 12♂/4♀); 2) 50 g/L cooked rice four ORS (n=16; $\bar{x}$ = 37.4 yrs; 10♂/6♀); or 3) 50 g/L uncooked HAMS + iso-osmolar glucose based ORS

(n=16; x̄ = 33.9 yrs; 11♂/5♀). All solutions had a similar osmolarity of ~327 mOsm per kg. Oral solution therapy was conducted according to World Health Organization treatment plan B, which is used for the management of mild to moderate dehydration and involves the administration of oral rehydration solution at a rate of 75 mL/kg body weight (to a maximum of 4 liters) in the first 4 hours. The primary end points were fecal weight (for every 12-hour period during the first 48 hours after enrollment) and length of time to the first formed stool. Statistical comparisons were conducted using 2-sample t-tests or Mann-Whitney U tests, as appropriate, with 2-tailed p values. Secondary endpoints included indirect measures of colonic fermentation efficiency *via* analyses of starch content of stools. All randomized subjects completed the study. Mean (±SD) fecal weights in the periods 12 to 24 hours, 24 to 36 hours, and 36 to 48 hours after enrollment were significantly lower in the HAMS group (2,206±1,158 g, 1,810 1,018 g, and 985±668 g) than the standard-therapy group (3,251±766 g, 2,621±1,149 g, and 2,498±1,080 g; p=0.01, p=0.04, and p=0.001, respectively). The mean duration of diarrhea was significantly shorter in the HAMS group (56.7±18.6 hours) than the standard-therapy group (90.9±29.8 hours, p=0.001). Fecal excretion of starch was higher in the HAMS group (32.6±30.4 g) than the standard-therapy group (11.7±4.1 g, p=0.002).

In a subsequent study by Ramakrishna and colleagues, the utility of HAMS as a substitute for glucose in hypo-osmolar ORS was evaluated in adults presenting to the Emergency Services of the Christian Medical College between May 2003 and June 2005 with acute severe dehydrating diarrhea. Fifty (50) adult males with severe watery diarrhea of less than three days' duration and moderate to severe dehydration were randomized to 1 of 2 groups administered 1) hypo-osmolar ORS (HO-ORS; n=25; x̄ = 33.9±12.0 yrs; n=10 *V. cholera* +) or 2) HO-ORS in which uncooked HAMS (50g/L) was substituted for glucose (HAMS-ORS; n=25; x̄ = 37.8±13.7 yrs; n=12 *V. cholera* +). All remaining therapy followed standard protocols for treatment of diarrhea in the clinic, including initial resuscitation using intravenous infusion and provision of 300 mg doxycycline. ORS intake in the first and second 12-hour intervals did not differ between the HAMS-ORS group (x̄ = 4,400 mL, 3,200 to 5,600 mL; x̄ = 2,200 mL, 1,450 to 2,800) and the HO-ORS group (x̄ = 4,400 mL, 3,000 to 6,100 and x̄ = 2,200 mL, 1,700 to 3,700 mL). Based on a concentration of 50 g/L, subjects consuming the HAMS-ORS are estimated to have consumed an average of 330 g of HAMS in the first 24 hours. In the second 24 hours ORS was significantly decreased in the HAMS group. Over the entire 48-hour treatment interval no differences in ORS intake were observed. The authors observed that that duration of diarrhea (ORS commencement to first formed stool) in hours was significantly shorter with HAMS-ORS (median 19, IQR 10-28) compared to HO-ORS (median 42, IQR 24-50) (Bonferroni adjusted P, Padj,0.001). Survival analysis (Kaplan-Meier) showed faster recovery from diarrhea in the HAMS-ORS group (p=0.001, log rank test). Total diarrhea fecal weight in grams (median, IQR) was not significantly lower in the HAMS-ORS group (2,190, 1,160-5,635) compared to HO-ORS (5,210, 2,095 to 12,190) (Padj=0.08). However, stool weight at 13 to 24 hours (280, 0 to 965 *vs*. 1,360, 405 to 2,985) and 25 to 48 hours (0, 0 to 360 *vs*. 1,080, 55 to 3,485) were

significantly lower in HAMS-ORS compared to HO-ORS group (Padj=0.048 and p=0.012, respectively). ORS intake after first 24 hours was lower in the HAMS-ORS group. Subgroup analysis of patients with culture isolates of *Vibrio cholerae* indicated similar significant differences between the treatment groups. No adverse effects related to HAMS use were observed, and no differences in the need for unscheduled intravenous fluid, or incidences of hyponatremia were observed between the groups. No differences in serum sodium, potassium or creatinine levels between groups were reported. The authors concluded that "Compared to HO-ORS, HAMS-ORS reduced *diarrhea duration by 55% and significantly reduced fecal weight after the first 12 hours of ORS therapy in adults with cholera-like diarrhea.*"

The effect of ORS solutions containing acetylated HAMS (HAMSA) or HAMS in treating dehydration in adults with severe acute gastroenteritis was evaluated by Pal *et al*. (2013). The study was conducted at Christian Medical College and Hospital (Vellore, India) in adults with diarrhea of less than 3 days' duration with moderate or severe dehydration. Patients with visible blood in stool, concomitant serious illness, sepsis, and coronary artery disease or stroke within the last six months, were excluded. Patients were randomly assigned to treatment with either uncooked HAMSA (50 g/L, mixed in reduced osmolarity ORS) or uncooked HAMS (50 g/L, mixed in reduced osmolarity ORS), keeping all other treatment standard. The primary outcome measure was diarrheal duration, defined as time from randomization to therapy to the first formed stool. Secondary outcome measures were total diarrheal stool output, the need for unscheduled intravenous fluids, and adverse events. One-hundred (100) adult patients were enrolled, 49 received HAMSA and 51 received HAMS. Twenty-four (24) patients (12 in each group) grew *Vibrio cholerae* in stool culture. The median duration of diarrhea was significantly shorter in the HAMSA group (24.5 hours) than in the HAMS group (36.5 hours, P=0.036). The median fecal weights from enrollment to formed stools were lower in the HAMSA group (1,770 g) than in HAMS group (2,360 g), but this was not statistically significant. There were no adverse events in the control or treatment arms. The need for unscheduled intravenous fluid was high in both the groups, as most of the patients had pre renal failure. None of the patients required dialysis. There was no hyponatremia in any of the patients in either control arm or treatment arm. The authors' concluded that the addition of starch acetate to ORS significantly shortened the duration of diarrhea in patients with acute infectious gastroenteritis (P=0.036). The apparent lack of effect of HAMSA on stool weight could be due to the fact that the study was not powered adequately to detect this secondary outcome. HAMSA is useful as an adjunct to ORS in the management of acute dehydrating diarrhea in adults.

6.4.5.2 Children with Rotavirus or Cholera

The use of HAMS as an adjunct to ORS in children with diarrhea was evaluated by Raghupathy *et al*. (2006). The study utilized a randomized controlled design and was conducted in a group of 183 children 6 months to 3 years of age presenting to the Christian Medical College (Vellore,

India) with acute mild to moderate diarrhea. Children were randomized to 1 of 2 groups provided the following ORS treatments: 1) standard treatment with G-ORS (n=91; $\bar{x}$ = 11.6±0.6 mo; n=30 Rotavirus +; n=6 *V. cholera* +) or 2) G-ORS with 50 g/L uncooked HAMS (n=87; $\bar{x}$ = 12.0.9±0.6 mo; n=28 Rotavirus; n=5 *V. cholera*). Children were treated according to standard practices at the hospital. Individuals presenting with server diarrhea were hydrated with intravenous solutions and then included in the study if intravenous therapy could be discontinued. Children were severe malnutrition were not included in the study. Stool weight and consistency were monitored serially until development of formed stool or development of treatment failure defined as either the need for unscheduled intravenous fluid therapy or diarrhea longer than 72 hours. The HAMS ORS solutions were well-tolerated by the infants did not induce vomiting or significant increase in diarrhea. Five of the subjects (n=2 HAMS-ORS; n=3 G-ORS) either did not receive the allocated intervention or were lost to follow up. In 178 remaining children (87 HAMS-ORS and 91 G-ORS) with evaluable data, time from enrollment to last unformed stool was significantly less in children receiving HAMS-ORS (median, 6.75 hours; 95% confidence interval, 4.27-9.22) than in children treated with G-ORS (12.80 hours, 8.69 to 16.91) (p=0.0292). Time to first formed stool was also significantly shorter in children receiving HAMS-ORS (median, 18.25 hours; 95% confidence interval, 13.09 to 23.41) compared with children receiving G-ORS (median, 21.50 hours; 95% confidence interval, 17.26 to 25.74) (p=0.0440). The total mean quantities of ORS consumed in the HAMS-ORS group was 79.6 mL (95 CI = 67.1 to 92.1 mL) and was similar to the G-ORS group at 87.3 mL (95 CI = 74.1 to 100.4). These intakes of ORS would correspond to acute daily intakes of between 3.4 to 4.6 g per child. There was a trend toward lower mean stool weight in first 24 hours (p=0.0752) as well as total diarrheal stool weight (p=0.0926) in patients in the HAMS group compared with the G-ORS group. Six children required unscheduled intravenous therapy in the HAMS-ORS group *vs*. 9 subjects in the G-ORS group (p=NS). Two subjects in each group had diarrhea persisting beyond 72 hours. The authors concluded that "*In children with acute diarrhea, the addition of amylase-resistant starch to glucose ORS significantly shortened duration of diarrhea compared with standard treatment*."

Alam *et al*. (2009) evaluated the safety of rapid intravenous therapy and comparative effects of three different ORS products in severely malnourished children with dehydrating cholera. The study was conducted in 175 subjects presenting to the Dhaka Hospital of the International Centre for Diarrhoeal Disease Research, Bangladesh from July 2001 to December 2004. All subjects enrolled in the study were *Vibrio cholera* positive. Children enrolled in the study were randomized to 1 of 3 ORS groups: 1) standard 90 mM glucose ORS (G-ORS; n=58; $\bar{x}$ = 21.17±12.3 mo; 26♂/32♀); 2) standard 90 mM glucose ORS + 50 g/L HAMS (G-ORS + HAMS; n=59; $\bar{x}$ = 28.6±13.42 mo; 34♂/25♀) or 3) 50 g/L rice-based ORS (Rice-ORS; n=58; $\bar{x}$ = 27.33±11.97 mo; 32♂/26♀). All intakes and outputs were quantified for each 6-hour period of the acute phase of the study. Vital signs (pulse, temperature, and respiration), and signs of dehydration or over-hydration were monitored every 6 hours. Before randomization 85% of the

children were re-hydrated with intravenous fluids and children were provided with antibiotic therapy in accordance with standard practice of the hospital. Children received vitamin A, folic acid, and elemental zinc as well multi-vitamin supplementation. The primary endpoints were recovery from diarrhea, and time to attain 80% of weight for length. Other endpoints measured included weight gain, stool output, vomit output, and urine output over 72 hours. ORS intake, water intake and milk formula intake, and need for unscheduled IV therapy also were monitored. The authors' reported a significant 35 to 45% reduction of stool weight during the first and second 24 hours among children receiving the rice-based ORS. There were no statistically significant differences between various outcomes among the subjects randomized to the glucose-ORS group vs. those provided the glucose+HAMS-ORS. The duration of diarrhea and the number of children receiving unscheduled intravenous therapy did not differ significantly between the groups. Time to attain 80% of weight for length also did not differ between groups.

Table 6.4.3-1 Summary of Clinical Studies Conducted to Evaluate the Safety and Efficacy of Oral Rehydration Solutions Containing High-Amylose Maize Starch

Study Design	Study Population	Test Articles	Dose and Duration of Intervention[a]	Key Results[b]	Reference
Adults					
Randomized and controlled Single-blinded: Subjects were not blinded due to visible differences in the test articles[a]	48 adolescent and adults with cholera diarrhea (acute, <72 h in duration) Ages 14 to 58 years	WHO-ORS (~327 mOSm/kg)[d] WHO-ORS plus 50 g/L HAMS (~327 mOSm/kg) WHO-ORS plus 50 g/L rice flour (~327 mOSm/kg)	• ORS administered according to WHO treatment plan B: 75 mL/kg bw during first 4 hours, to a maximum of 4 L, and continuing as necessary • Fecal collection continued for 48 h, and the subjects were hospitalized until consistency of stool returned to normal	• All subjects completed the study • ORS+HAMS SS↓ fecal weight from 12-24, 24-36, and 36-48 h compared to ORS only • ORS+HAMS SS ↓ fecal weight from 36-48h compared to ORS+rice flour • Time to form first stool (*i.e.*, duration of diarrhea) was SS ↓ in ORS+HAMS compared to ORS only or ORS+rice flour • Amount of starch excreted in feces was SS ↑ in ORS+HAMS compared to ORS only or ORS+rice flour	Ramakrishna *et al.*, 2000
Randomized, and controlled Single-blinded: Subjects were not blinded due to visible differences in the test articles[a]	50 adult males with cholera or cholera-like diarrhea (<3 days) and moderate to severe dehydration (subgroup with cholera, n = 22) Ages: 12 to 65 Mean±SD: 42.6±12.0 (HAMS-ORS); 37.8±13.7 (HO-ORS)	WHO hypo-osmolar ORS (HO-ORS), 245 mOsm/kg with 13.5 g/L glucose HO-ORS where glucose was substituted by 50 g/L HAMS (HAMS-HO-ORS), 170 mOsm/kg	• ORS administered at 200 mL/hour, and 200 mL after each loose stool until stool becomes formed • Subjects were hospitalized for 48 hours or until stool consistency was reported as "normal" • 6,200 to 13,100 mL HO-ORS consumed over 48 hours (mean = 10,000 mL) • 5,700 to 10,000 mL HAMS-HO-ORS consumed over 48 hours (mean = 7,200 mL) • NSD in volume of ORS consumed between groups over 48 hours	• All subjects completed the study and none required dialysis for renal failure • NSD in incidence of hyponatremia, or in serum levels of K^+ and creatinine between groups • NSD in unscheduled i.v. therapy between groups, and only 1 subject in HO-ORS had diarrhea persisting longer than 48 h • HAMS-ORS SS ↓ time to form first stool and had faster recovery from diarrhea • NSD in total fecal weight (but trend towards lower values in HAMS-ORS group) • Fecal weight during 12-24 h, and 24-48 h was SS ↓ in HAMS-HO-ORS compared to HO-ORS • HAMS-ORS SS improved recovery time from diarrhea in patients with or without positive *Vibrio* cultures	Ramakrishna *et al.*, 2008

Table 6.4.3-1 Summary of Clinical Studies Conducted to Evaluate the Safety and Efficacy of Oral Rehydration Solutions Containing High-Amylose Maize Starch

Study Design	Study Population	Test Articles	Dose and Duration of Intervention[a]	Key Results[b]	Reference
Randomized, double-blind, controlled	100 adult with watery diarrhea (<3 days) and moderate to severe dehydration (subgroup with cholera)	WHO reduced osmolarity ORS containing 50 g/L of HAMS, or 50 g/L of acetylated HAMS (HAMSA)	Not available?	• No adverse events reported in either group • Unscheduled i.v. fluid required in both groups due to high incidence of pre-renal failure, but none required dialysis • No hyponatremia observed in either arm • Diarrhea duration SS↓ in HAMSA compared to HAMS • NSD in total fecal weight between groups	Pal *et al.*, 2013 (abstract only)
Children					
Randomized, and controlled Single-blinded: Subjects were not blinded due to visible differences in the test articles[a]	183 children with acute watery diarrhea and clinical diagnosis of dehydration (subgroup with cholera or rotavirus, n=76) Ages 6 months to 3 years (mean bw = 8.2±0.2 kg)	WHO-ORS (311 mOsm/kg; 20 g/L of glucose) WHO-ORS plus 50 g/L HAMS (osmolarity of the final solution not reported)	• ORS administered according to WHO treatment plan B: 75 mL/kg bw during first 4 hours, to a maximum of 4 L, and continuing as necessary • Study ended when there was a formed stool or when there was "treatment failure" (unscheduled i.v. fluid therapy or persistence of diarrhea beyond 72h) • Mean HAMS-ORS consumed: 79.6 mL/kg bw, or ~654 mL (95% CI: 67.1 to 92.1 mL/kg bw, or ~550 to 755mL) • Mean WHO-ORS consumed: 87.3 mL/kg bw, or ~716 mL (95% CI: 74.1 to 100.4 mL/kg bw, or ~608 to 823 mL)	• HAMS-ORS was well tolerated and did not induce vomiting or significant ↑ diarrhea • NSD in unscheduled i.v. therapy or persistence of diarrhea beyond 72 hr between groups • NSD in mean stool output during the first 24 h or total stool output between groups (but trend towards lower values in HAMS-ORS group) • HAMS-ORS SS ↓ time to form first stool *vs.* ORS only • NSD of HAMS-ORS in time to form first stool or stool output in subgroup analysis of those with pathogen infection	Raghupathy *et al.*, 2006

Table 6.4.3-1 Summary of Clinical Studies Conducted to Evaluate the Safety and Efficacy of Oral Rehydration Solutions Containing High-Amylose Maize Starch

Study Design	Study Population	Test Articles	Dose and Duration of Intervention[a]	Key Results[b]	Reference
Randomized and controlled Study (all people involved in the study) was not blinded due to visible differences in the test articles	175 severely malnourished children with cholera diarrhea (acute, <48 h in duration) Ages 6 to 60 months	WHO-ORS (305 mOsm/L; 16 g/L of glucose) WHO-ORS plus 50 g/L of HAMS (305 mOsm/L) Rice-based ORS (215 mOsm/L)	• All subjects were defined as having "some dehydration" before test articles were administered[c] • Subjects were administered ORS at 100 mL/kg bw for a 6-hr period • The same solution was given at 5 to 10 mL/kg bw after each watery stool to match ongoing stool losses, until diarrhea stopped	• None of the children developed signs of overhydration, cardiac failure, hypoglycemia, severe hypo- or hyper-kalemia, or severe hypo- or hyper-natremia • No mortalities observed during the study • NSD in number of children receiving unscheduled i.v. therapy • In children given HAMS+ORS *vs.* ORS o NSD in body weight gain over 72 h o NSD in stool, vomit, and urine output o NSD in the duration of diarrhea	Alam *et al.*, 2009
				• Stool from subset of children were analyzed for fecal bacteria and SCFA concentration • NSD in total SCFA or individual SCFA (acetate, propionate, butyrate, or valerate) in HAMS+ORS *vs.* ORS only • NSD in number of bacteria or type of bacteria in stool samples in HAMS+ORS *vs.* ORS only	Monira *et al.*, 2009

HAMS = high-amylose maize starch; i.v. = intravenous; mOsm =milli-osmolar; NSD = no significant differences; ORS = oral rehydration solution; SS = statistically significant; WHO = World Health Organization

[a] The volume of ORS consumed by the subjects are presented in the table if they were provided in the publication.

[b] Due to the nature of the suspensions formed by HAMS and other carbohydrates (*i.e.*, rice flour) in solution, in comparison to the standard glucose-based ORS, it was not possible to ensure that the patients and caregivers were blinded to the nature of the test article. However, the subjects would not be able to differentiate between an ORS containing rice flour compared to HAMS. The investigator responsible for the collection, weighing and analysis of feces were blinded to the intervention received.

[c] Children who were "severely dehydrated" at enrollment were rehydrated by intravenous fluid therapy until they have recovered to "some dehydration" state. Children with "some dehydration" upon enrollment received the assigned ORS within 1 hour, while children with "severe dehydration" upon enrollment received the assigned ORS within 6 hours following i.v. rehydration. Dehydration was defined using a slightly modified version of the WHO guidelines, whereby "some dehydration" was considered as presence of at least 2 signs or symptoms (irritable/less active*, sunken eyes, dry mucosa, thirst*, reduced skin turgor*) with at least 1 of the key indicators marked by asterisk. Severe dehydration was defined as the presence of signs of some dehydration plus at least 1 of the following key signs: lethargy/coma, inability to drink but not refusal to drink, or uncountable/absent radial pulse.

[d] Glucose content is not stated, but the WHO-recommended glucose-based ORS, which typically contains 20 g/L glucose, was used in this study.

6.5 Summary

High-amylose maize starch is a dietary carbohydrate with a long history of consumption in the food supply. It is a naturally occurring form of resistant starch that is incompletely digested in the upper gastrointestinal tract, but rather, a fraction reaches the colon where it is fermented by the resident microflora to release gases and SCFA. Similar to other forms of incompletely digested but fermentable forms of carbohydrates, the primary safety concern associated with consumption of large amounts of these substances is the potential for adverse gastrointestinal effects. For example, the gases produced from fermentation may result in flatulence, bloating, and abdominal discomforts, and the undigested carbohydrates or unabsorbed end products of fermentation (*i.e.*, SCFA) may potentially cause osmotic diarrhea (Grabitske and Slavin, 2009). Nevertheless, it has been suggested that since the colon has a high capacity for fermentation of resistant starches, ingestion of large amount does not appear to pose adverse gastrointestinal effects among well-nourished individuals living in regions with good personal hygiene (Annison and Topping, 1994; Topping and Clifton, 2001). Grabitske and Slavin (2009) has indicated the acceptable daily intakes of resistant starches (RS_2 and RS_3) to be 45 g/day, based on the increased incidence of flatulence at higher doses. Furthermore, resistant starches are considered to have high laxation threshold, with only rare cases of diarrhea reported even at doses as high as 80 g/day (Grabitske and Slavin, 2009).

Infections with enterotoxigenic organisms (*e.g.*, *V. cholerae*, enterotoxigenic *E. coli*, rotaviruses) are the most common cause of acute diarrhea; the enterotoxins secreted by these organisms cause disruptions in the electrolyte transport systems in enterocytes through cAMP/cGMP dependent mechanisms, thereby resulting in massive water losses (Kopic and Geibel, 2010; Binder *et al.*, 2014). The standard glucose-based ORS that are currently endorsed for managing dehydration associated with diarrhea help restore fluid and electrolyte balance by stimulating glucose and sodium absorption in the small intestines (in a cAMP-independent manner), which in turns promote water absorption due to the osmotic gradient generated (CDC, 2003; Kelly and Nadeau, 2004; Binder *et al.*, 2014). It has been proposed that resistant starches such as HAMS may have utility as an adjunct and/or replacement for glucose in ORS, as these carbohydrates can serve as a low-osmotic source of digestible glucose, while the end-products of microbial fermentation (*i.e.*, SCFA) may further promote the absorption of electrolytes and water within the colon (Binder *et al.*, 2014). Cereal-based ORS (such as rice-based ORS), which would work by similar mechanisms, are known to be safe and efficacious in restoring hydration during acute diarrhea (Gregorio *et al.*, 2009). Furthermore, whole-gut perfusion studies conducted in rats have demonstrated that ORS containing 50 g/L HAMS significantly increased net absorption of electrolytes (*i.e.*, Na^+, K^+, and Cl^-) and water, while the secretion of bicarbonate was significantly decreased, when compared to perfusion with standard glucose-based ORS (Subramanya *et al.*, 2006).

Multiple randomized controlled trials demonstrating that the use of HAMS in ORS is safe and suitable have been published. Two of the studies were conducted in young children (less than 5 years of age) (Raghupathy *et al.*, 2006; Alam *et al.*, 2009), while 3 of the studies were in adults (considered as age 12 or older) (Ramakrishna *et al.*, 2000, 2008; Pal *et al.*, 2013). Two of the studies were conducted specifically in children or adults with cholera diarrhea (Ramakrishna *et al.*, 2000; Alam *et al.*, 2009), while a subset of subjects had stool samples positive for *Vibrio cholerae* in the other studies (Raghupathy *et al.*, 2006; Ramakrishna *et al.*, 2008; Pal *et al.*, 2013). In 3 out of the 5 studies, HAMS was administered as an adjunct to the standard iso-osmolar glucose-based ORS recommended by the WHO (Ramakrishna *et al.*, 2000; Raghupathy *et al.*, 2006; Alam *et al.*, 2009). Only 1 study has been conducted that tested the effect of ORS where glucose was completely replaced by HAMS, when compared to the effects of the same standard hypo-osmolar glucose-based ORS, among adults with acute cholera and non-cholera diarrhea (Ramakrishna *et al.*, 2008). In this study, the volume of HAMS-containing ORS consumed ranged from 5,700 to 10,000 mL, which is equivalent to intakes of approximately 500 g HAMS over the course of 48 hours (*i.e.*, approximately 125 g/day). One recent study compared the effect of ORS containing HAMS with an ORS containing acetylated HAMS (Pal *et al.*, 2013).

Overall, administration of HAMS at a use level of 50 g/L, regardless of whether it was added as an adjunct to the standard glucose-based ORS or as a complete replacement for glucose in ORS, was well tolerated without any adverse effects observed (*e.g.*, number of mortalities, the need for unscheduled intravenous fluid therapy, worsening of diarrhea, increase in vomiting, development of persistent diarrhea, or hyponatremia and other disruptions in electrolyte levels). There is also some evidence that HAMS, when added as an adjunct to standard glucose-based ORS therapy, significantly decreased fecal weight (*i.e.*, decreased water loss in stool) as well as decreased time to form first stool (*i.e.*, decreased duration of diarrhea), when compared to administration of glucose-based ORS alone in children (Raghupathy *et al.*, 2006). However, these findings were not observed among children with cholera diarrhea (Alam *et al.*, 2009). Fecal weight and time to form first stool were significantly decreased in adults with cholera diarrhea who were administered HAMS as an adjunct to standard glucose-based ORS, compared to administration of glucose-based ORS alone (Ramakrishna *et al.*, 2000). Similarly, fecal weight and time to form first stool were also significantly decreased in adults with both cholera and non-cholera diarrhea who were administered ORS where glucose was completely replaced by HAMS (Ramakrishna *et al.*, 2008).

Data from a large number of studies conducted with “glucose polymer-based” ORS where carbohydrates from cereal sources (*e.g.*, wheat, rice) are used as the glucose source, also corroborate the safe use of HAMS in ORS. In these polymer-based ORS, the glucose is slowly digested and released in the small intestines, thereby aiding the re-absorption of water and electrolytes without adding a large osmotic load to small intestine (Gregorio *et al.*, 2009). As

discussed, the resistant starch present in some of these carbohydrate sources also may promote water absorption in the colon following fermentation by the indigenous microflora (Binder *et al.*, 2014). Gregorio *et al.* (2009) has conducted a meta-analysis of randomized controlled studies that compared the effects of polymer-based ORS with glucose-based ORS in subjects (both children and adults) with acute watery diarrhea (both cholera and non-cholera associated). Studies were included if they administered ORS in which glucose was replaced by common dietary sources of glucose polymers (*i.e.*, rice, wheat, sorghum, and high-amylose maize[6]). It was concluded that subjects administered polymer-based ORS had fewer unscheduled intravenous fluid therapy compared to those administered the glucose-based ORS. Administration of polymer-based ORS was associated with a reduced duration of diarrhea among adults positive for *V. cholerae* compared to those administered a standard glucose-based ORS, though this finding was not reported in adults with non-cholerae or mixed pathogens, or in children. The incidence of adverse effects (*e.g.*, number of unscheduled use of intravenous fluids, number of episodes of vomiting, incidence of hyponatremia, hypokalemia, or development of persistent diarrhea) were similar between those receiving polymer-based ORS compared to standard glucose-based ORS.

Overall, the clinical studies conducted to date suggest that addition of HAMS to ORS is safe and suitable. The majority of the studies have investigated the effects of HAMS when used as an adjunct to standard glucose-based ORS, rather than as a replacement for glucose in ORS. Nevertheless, replacement of glucose by HAMS in ORS did not result in any adverse effect, and may even reduce stool output and duration of diarrhea, compared to standard glucose-based ORS in adults with acute diarrhea (both cholera and non-cholera related) (Ramakrishna *et al.*, 2008). Furthermore, other "glucose-polymer" based ORS that contains cereal carbohydrates as the glucose source have been shown to be at least as safe and efficacious as standard glucose-based ORS, indicating that the use of HAMS would be acceptable in individuals (both children and adults) with acute diarrhea of any etiology.

7.0 CONCLUSIONS

Based on available data and information presented herein, Yale has determined that high-amylose maize starch, meeting appropriate food grade specifications and manufactured in accordance with current Good Manufacturing Practices (cGMP), is safe and suitable for addition to ORS products at a use level of 50 g/L.

[6] Although studies were included in the meta-analysis only if they administered ORS in which glucose was replaced by a glucose-polymer, the study by Ramakrishna *et al.* (2000) was also included even though HAMS was administered as an adjunct to glucose-based ORS. Notably, the study by Raghupathy *et al.*, 2006 was excluded from the meta-analysis specifically because HAMS was added to a glucose-based ORS, rather than being used in place of glucose.

Yale also has concluded that high-amylose maize starch, meeting appropriate food grade specifications and manufactured in accordance with current Good Manufacturing Practices (cGMP), is Generally Recognized as Safe (GRAS) as an ingredient for addition to ORS at a use level of 50 g/L in ORS, based on scientific procedures.

8.0 REFERENCES

AAP (2004). Managing acute gastroenteritis among children: oral rehydration, maintenance, and nutritional therapy (American Academy of Pediatrics). Pediatrics 114(2):507. Available at: http://pediatrics.aappublications.org/content/114/2/507.full.

Ackerson CW (1961). High-amylose corn in a diet for day-old chicks. Feed Age 11(7):25-27. Cited In: Sandstedt et al., 1962 [Ref. #1].

Alam NH, Islam S, Sattar S, Monira S, Desjeux JF (2009). Safety of rapid intravenous rehydration and comparative efficacy of 3 oral rehydration solutions in the treatment of severely malnourished children with dehydrating cholera. J Pediatr Gastroenterol Nutr 48(3):318-327.

Annison G, Topping DL (1994). Nutritional role of resistant starch: Chemical structure vs physiological function. Annu Rev Nutr 14:297-320.

Asp NG, Björck I (1992). Resistant starch. Trends Food Sci Technol 3:111-114.

Asp N-G, van Amelsvoort JMM, Hautvast JGAJ (1996). Nutritional implications of resistant starch. Nutr Res Rev 9:1-31.

Atia AN, Buchman AL (2009). Oral rehydration solutions in non-cholera diarrhea: a review [including "Continuing medical education questions: October 2009"]. Am J Gastroenterol 104(10):2596-2605.

Ball JM, Tian P, Zeng CQ, Morris AP, Estes MK (1996). Age-dependent diarrhea induced by a rotaviral nonstructural glycoprotein. Science 272(5258):101-104. Cited In: Kopic and Geibel, 2010 [Ref. #203].

BeMiller JN (1973). Chapter XXIV – Starch amylase. In: Whistler R, BeMiller JN, editor. *Industrial Gum: Polysaccharides and Their Derivatives, 2nd edition*. New York (NY): Academic Press, pp. 545-566 [See p. 546]. Available at: http://books.google.ca/books?id=VWLae16j8XEC&lpg=PA546&ots=8_lHRJZX4Z&dq=amylomaize%2050%20to%2080%25%20amylose&pg=PA546#v=onepage&q=amylomaize%2050%20to%2080%25%20amylose&f=false.

BeMiller JN (2009). One hundred years of commercial food carbohydrates in the United States. J Agric Food Chem 57(18):8125-8129.

Binder HJ (2010). Role of colonic short-chain fatty acid transport in diarrhea. Annu Rev Physiol 72:297-313.

Binder HJ, Ramakrishna BS (2004). Resistant starch-An adjunct to oral rehydration solution: Not yet ready for prime time. J Pediatr Gastroenterol Nutr 39(4):325-327.

Binder HJ, Brown I, Ramakrishna BS, Young GP (2014). Oral rehydration therapy in the second decade of the twenty-first century. Curr Gastroenterol Rep 16(3):376 [8 p.]. DOI 10.1007/s11894-014-0376-2.

Bird AR, Vuaran M, Brown I, Topping DL (2007). Two high-amylose maize starches with different amounts of resistant starch vary in their effects on fermentation, tissue and digesta mass accretion, and bacterial populations in the large bowel of pigs. Br J Nutr 97(1):134-144.

Bird AR, Conlon MA, Christophersen CT, Topping DL (2010). Resistant starch, large bowel fermentation and a broader perspective of prebiotics and probiotics. Benef Microbes 1(4):423-431.

Borchers R (1962). A note on the digestibility of the starch of high-amylose corn by rats. Cereal Chem 39(2):145-146.

Brown IL (1994). High amylose starches - new developments in human nutrition. Proc Nutr Soc Austral 18:33-39.

Caderni G, Luceri C, Lancioni L, Dolara P (1996). Dietary sucrose, glucose, fructose, and starches affect colonic functions in rats. Nutr Cancer 25(2):179-186.

CDC (2003). *Managing Acute Gastroenteritis Among Children Oral Rehydration, Maintenance, and Nutritional Therapy*. (Morbidity and Mortality Weekly Report (MMWR), vol 52, no RR-16). Atlanta (GA): Centers for Disease Control and Prevention (CDC). Available at: http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5216a1.htm.

Clarke JM, Bird AR, Topping DL, Cobiac L (2007). Excretion of starch and esterified short-chain fatty acids by ileostomy subjects after the ingestion of acylated starches. Am J Clin Nutr 86(4):1146-1151.

Cotran RS, Kumar V, Robbins SL, editors (1999). *Robbins Pathologic Basis of Disease, 6th edition*. Philadelphia (PA): WB Saunders.

Cummings JH, Englyst HN (1995). Gastrointestinal effects of food carbohydrate. Am J Clin Nutr 61(4, Suppl.):938S-945S.

de Sales PM, de Souza PM, Simeoni LA, de Oliveira Magalhães P, Silveira D (2012). α-Amylase inhibitors: a review of raw material and isolated compounds from plant source. J Pharm Pharm Sci 15(1):141-183.

De Schrijver R, Vanhoof K, Vande Ginste J (1999). Effect of enzyme resistant starch on large bowel fermentation in rats and pigs. Nutr Res 19(6):927-936.

den Besten G, van Eunen K, Groen AK, Venema K, Reijngoud DJ, Bakker BM (2013). The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. J Lipid Res 54(9):2325-2340.

Dong Y, Zeng CQ, Ball JM, Estes MK, Morris AP (1997). The rotavirus enterotoxin NSP4 mobilizes intracellular calcium in human intestinal cells by stimulating phospholipase C-mediated inositol 1,4,5-trisphosphate production. Proc Natl Acad Sci U S A 94(8):3960-3965. Cited In: Kopic and Geibel, 2010 [Ref. #202].

Dysseler P, Hoffem D (1995). Estimation of resistant starch intake in Europe. In: Asp N-G, van Amelsvoort JGAJ, Hautvast JGAJ, editors. *Proceedings of the Concluding [3rd] Plenary Meeting of EURESTA - Including the Final Reports of the Working Groups*, Apr. 13-17, 1994, La Londe - Les Maures, France. (European FLAIR Concerted Action no 11 on Physiological Implications of the Consumption of Resistant Starch in Man). Wageningen, The Netherlands: EURESTA/FLAIR (Commission of the European Communities Directorate General XII, Science, Research and Development, Food-Linked Agro-Industrial Research/FLAIR), pp. 84-86. Also Cited In: Asp et al., 1996. [As Dyssler and Hoffem, 1995c].

ESPGAN Working Group (1992). Recommendations for composition of oral rehydration solutions for the children of Europe. Report of an ESPGAN Working Group. J Pediatr Gastroenterol Nutr 14(1):113-115.

FAO/WHO (1998). Dietary fibre and resistant starch analysis. In: *Carbohydrates in Human Nutrition*. Report of a Joint FAO/WHO Expert Consultation, Apr. 14-18, 1997, Rome, Italy. (FAO Food and Nutrition Paper no. 66). Rome, Italy: Food and Agriculture Organization of the United Nations (FAO) / Geneva, Switz.: World Health Organization (WHO). Available at: http://www.fao.org/docrep/W8079E/W8079E00.htm.

FAO/WHO (2009). *Principles and Methods for the Risk Assessment of Chemicals in Food: Annex 2. Dose Conversion Table.* (Environmental Health Criteria 240, Annex 2). Geneva, Switz: Food and Agriculture Organization of the United Nations (FAO) / World Health Organization (WHO), International Programme on Chemical Safety (IPCS). Available at: http://whqlibdoc.who.int/ehc/WHO_EHC_240_14_eng_Annex2.pdf.

Farthing M, Salam MA, Lindberg G, Dite P, Khalif I, Salazar-Lindo E et al. (2013). Acute diarrhea in adults and children: a global perspective. J Clin Gastroenterol 47(1):12-20.

FASEB (1979). *Evaluation of the Health Aspects of Starch and Modified Starches as Food Ingredients*. (PB80-128804; SCOGS-115; FDA/BF- 80/44). Prepared by Bethesda (MD): Federation of American Societies for Experimental Biology (FASEB) for Washington (DC): Food and Drug Administration, U.S. (FDA), Bureau of Foods.

FCC (2014). Food starch, unmodified. In: *Food Chemicals Codex, 9th edition*. Rockville (MD): United States Pharmacopeial Convention (USP), pp. 499-501.

Ferguson LR, Tasman-Jones C, Englyst H, Harris PJ (2000). Comparative effects of three resistant starch preparations on transit time and short-chain fatty acid production in rats. Nutr Cancer 36(2):230-237.

Filer LJ Jr (1988). Modified food starch—an update. J Am Diet Assoc 88(3):342-344.

Fujita S, Glover DV, Okuno K, Fuwa H (1989). *In vitro* and *in vivo* digestion of high-amylose type starch granules. Starke [Starch] 41(6):221-224.

Gajda M, Flickinger EA, Grieshop CM, Bauer LL, Merchen NR, Fahey GC Jr (2005). Corn hybrid affects in vitro and in vivo measures of nutrient digestibility in dogs. J Anim Sci 83(1):160-171.

Gee JM, Faulks RM, Johnson IT (1991). Physiological effects of retrograded, alpha-amylase-resistant cornstarch in rats. J Nutr 121(1):44-49.

Goldring JM (2004). Resistant starch: safe intakes and legal status. J AOAC Int 87(3):733-739.

Grabitske HA, Slavin JL (2008). Low-digestible carbohydrates in practice. J Am Diet Assoc 108(10):1677-1681.

Grabitske HA, Slavin JL (2009). Gastrointestinal effects of low-digestible carbohydrates. Crit Rev Food Sci Nutr 49(4):327-360.

Granfeldt YE, Drews AW, Bjorck IM (1993). Starch bioavailability in arepas made from ordinary or high amylose corn: Concentration and gastrointestinal fate of resistant starch in rats. J Nutr 123(10):1676-1684.

Gregorio GV, Gonzales MLM, Dans LF, Martinez EG (2009). Polymer-based oral rehydration solution for treating acute watery diarrhoea. In: *Cochrane Database of Systematic Reviews*, Issue 2. Art. No.: CD006519. DOI: 10.1002/14651858.CD006519.pub2.

Grubben MJAL, Van Den Braak CCM, Essenberg M, Olthof M, Tangermana, Katan MB et al. (2001). Effect of resistant starch on potential biomarkers for colonic cancer risk patients with colonic adenomas: a controlled trial. Dig Dis Sci 46(4):750-756.

Guarino A, Albano F, Ashkenazi S, Gendrel D, Hoekstra JH, Shamir R et al. (2008). European Society for Paediatric Gastroenterology, Hepatology, and Nutrition/European Society for Paediatric Infectious Diseases evidence-based guidelines for the management of acute gastroenteritis in children in Europe. J Pediatr Gastroenterol Nutr 46(Suppl. 2):S81-S122.

Hall JE (2011). Chapter 65. Physiology of gastrointestinal disorders. In: *Guyton and Hall Textbook of Medical Physiology, 12th edition*. Philadelphia (PA): W.B. Saunders Company, Elsevier.

Hallauer AR (2004). Specialty corns. In: Smith CW, Betrán J, Runge ECA, editors. *Corn: Origin, History, Technology, and Production*. Hoboken (NJ): John Wiley, pp. 897-932 [See p. 922]. Available at: http://books.google.ca/books?id=eDJ3NjHh8H8C&lpg=PA922&ots=-eqgC1lLS_&dq=food%20application%20of%20high%20amylose%20corn%20starch&pg=PA922#v=onepage&q=food%20application%20of%20high%20amylose%20corn%20starch&f=false.

Hammer HF, Santa Ana CA, Schiller LR, Fordtran JS (1989). Studies of osmotic diarrhea induced in normal subjects by ingestion of polyethylene glycol and lactulose. J Clin Investig 84(4):1056-1062. Cited In: Binder, 2010 [Ref. #61].

Havenaar R (2011). Intestinal health functions of colonic microbial metabolites: a review. Benef Microbes 2(2):103-114.

Heijnen ML, van Amelsvoort JM, Deurenberg P, Beynen AC (1996). Neither raw nor retrograded resistant starch lowers fasting serum cholesterol concentrations in healthy normolipidemic subjects. Am J Clin Nutr 64(3):312-318.

Heijnen M-LA, Van Amelsvoort JMM, Deurenberg P, Beynen AC (1998). Limited effect of consumption of uncooked (RS2) or retrograded (RS3) resistant starch on putative risk factors for colon cancer in healthy men. Am J Clin Nutr 67(2):322-331.

Hijova E, Chmelarova A (2007). Short chain fatty acids and colonic health. Bratisl Lek Listy 108(8):354-358.

Hodges K, Gill R (2010). Infectious diarrhea: Cellular and molecular mechanisms. Gut Microbes 1(1):4-21.

Holtug K, Clausen MR, Hove H, Christiansen J, Mortensen PB (1992). The colon in carbohydrate malabsorption: short-chain fatty acids, pH, and osmotic diarrhoea. Scand J Gastroenterol 27(7):545-552.

Hylla S, Gostner A, Dusel G, Anger H, Bartram HP, Christl SU et al. (1998). Effects of resistant starch on the colon in healthy volunteers: Possible implications for cancer prevention. Am J Clin Nutr 67(1):136-142.

Ikai M, Kasaoka S, Oh-Hashi A, Morita T, Kiriyama S (1997). Digestibility of high-amylose corn starch in ileorectostomized and normal rats. Nippon Eiyo Shokuryo Gakkaishi [J Jap Soc Nutr Food Sci] 50(3):209-216.

IOM (2005). Dietary, functional, and total fiber. In: *Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein and Amino Acids (Macronutrients)*. (National Academy of Sciences (NAS), Institute of Medicine (IOM), Food and Nutrition Board (FNB), Panel on Micronutrients, Panel on the Definition of Dietary Fiber, Subcommittee on Upper Reference Levels of Nutrients, Subcommittee on Interpretation and Uses of Dietary Reference Intakes, Standing Committee on the Scientific Evaluation of Dietary Reference Intakes). Washington (DC): National Academy Press (NAP), pp. 339-421. Available at: http://www.nap.edu/openbook.php?record_id=10490&page=339.

Ito T, Saito K, Sugawara M, Mochida K, Nakakuki T (1999). Effect of raw and heat-moisture-treated high-amylose corn starches on the process of digestion in the rat digestive tract. J Sci Food Agric 79(9):1203-1207.

JECFA (1969). Amylose and amylopectin. In *Toxicological Evaluation of Some Food Colours, Emulsifiers, Stabilizers, Anti-Caking Agents and Certain Other Substances*. 3th Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), May 27-June 4, 1969. (FAO Nutrition Meetings Report Series, no 46A; WHO/FOOD ADD/70.36). Rome, Italy: Food and Agriculture Organization of the United Nations (FAO) / Geneva, Switz.: World Health Organization (WHO). Available at: http://www.inchem.org/documents/jecfa/jecmono/v46aje21.htm.

JECFA (1974). Amylose and amylopectin. In *Toxicological Evaluation of Some Food Additives Including Anticaking Agents, Antimicrobials, Antioxidants, Emulsifiers and Thickening Agents*. 17th Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), June 25-July 4, 1973, Geneva, Switz. (WHO Food Additives Series no 5). Rome, Italy: Food and Agriculture Organization of the United Nations (FAO). Available at: http://www.inchem.org/documents/jecfa/jecmono/v05je65.htm.

JECFA (1982). *Evaluation of Certain Food Additives and Contaminants*. 26th Report of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), Apr. 19-28, 1982, Rome, Italy. (WHO Technical Report Series, no 683). Geneva, Switz.: World Health Organization (WHO). Available at: http://whqlibdoc.who.int/trs/WHO_TRS_683.pdf.

Jenkins DJ, Vuksan V, Kendall CW, Wursch P, Jeffcoat R, Waring S et al. (1998). Physiological effects of resistant starches on fecal bulk, short chain fatty acids, blood lipids and glycemic index. J Am Coll Nutr 17(6):609-616.

Jiang H, Jane J-L (2013). Type 2 resistant starch in high-amylose maize starch and its development. In: Shi Y-C, Maningat CC, editors. *Resistant Starch: Sources, Applications and Health Benefits*. Chichester, UK: IFT Press/John Wiley & Sons, Ltd., pp. 23-42.

Kaiser P, Borte M, Zimmer KP, Huppertz HI (2012). Complications in hospitalized children with acute gastroenteritis caused by rotavirus: a retrospective analysis. Eur J Pediatr 171(2):337-345.

Kasaoka S, Morita T, Ikai M, Oh-Hashi A, Kiriyama S (1999a). Effect of high-amylose cornstarch on faecal excretion in rats. Nippon Eiyo Shokuryo Gakkaishi [J Jap Soc Nutr Food Sci] 52(5):263-270.

Kasaoka S, Morita T, Hase K, Kiriyama S (1999b). Psyllium regulates the colonic fermentation of high-amylose cornstarch and increases butyrate concentration in the distal colon and feces of rats. Nippon Eiyo Shokuryo Gakkaishi [J Jap Soc Nutr Food Sci] 52(6):365-372.

Kelly DG, Nadeau J (2004). Oral rehydration solution: a “low-tech” oft neglected therapy [Nutrition Issues in Gastroenterology, Series #21]. Pract Gastroenterol 21(10):51-62. Available at: http://www.oley.org/documents/ORS%20Article%20-%20Dr.%20Kelly.pdf.

Kendall CWC, Jenkins DJA, Emam A (2003). Assessment of resistant starch tolerance: a dose response study. Ann Nutr Metab 47(6):636 [abstract S636].

Kishida T, Nogami H, Himeno S, Ebihara K (2001). Heat moisture treatment of high amylose cornstarch increases its resistant starch content but not its physiologic effects in rats. J Nutr 131(10):2716-2721.

Kopic S, Geibel JP (2010). Toxin mediated diarrhea in the 21 century: the pathophysiology of intestinal ion transport in the course of ETEC, *V. cholera* and rotavirus infection. Toxins (Basel) 2(8):2132-2157.

Lajvardi A, Mazarin GI, Gillespie MB, Satchithanandam S, Calvert RJ (1993). Starches of varied digestibilities differentially modify intestinal function in rats. J Nutr 123(12):2059-2066.

Le Leu RK, Brown IL, Hu Y, Young GP (2003). Effect of resistant starch on genotoxin-induced apoptosis, colonic epithelium, and lumenal contents in rats. Carcinogenesis 24(8):1347-1352.

Lecona E, Olmo N, Turnay J, Santiago-Gonzalez A, Lopez de Silanes I, et al. (2008). Kinetic analysis of butyrate transport in human colon adenocarcinoma cells reveals two different carrier-mediated mechanisms. Biochem J 409(1):311-320. Cited In: Binder, 2010 [Ref. #38].

Liu H, Ramsden L, Corke H (1997). Physical properties and enzymatic digestibility of acetylated *ae*, *wx*, and normal maize starch. Carbohydr Polym 34(4):283-289.

Livesey G (2001). Tolerance of low-digestible carbohydrates: a general view. Br J Nutr 85(Suppl. 1):S7-S16.

Lopez HW, Levrat-Verny MA, Coudray C, Besson C, Krespine V, Messager A et al. (2001). Class 2 resistant starches lower plasma and liver lipids and improve mineral retention in rats. J Nutr 131(4):1283-1289.

Maki KC, Pelkman CL, Finocchiaro ET, Kelley KM, Lawless AL, Schild AL et al. (2012). Resistant starch from high-amylose maize increases insulin sensitivity in overweight and obese men. J Nutr 142(4):717-723.

Martínez I, Kim J, Duffy PR, Schlegel VL, Walter J (2010). Resistant starches types 2 and 4 have differential effects on the composition of the fecal microbiota in human subjects. PLoS One 5(11):e15046. doi: 10.1371/journal.pone.0015046.

McClean P, Weaver LT (1993). Ontogeny of human pancreatic exocrine function. Arch Dis Child 68(Spec No. 1):62-65.

Miyauchi S, Gopal E, Fei YJ, Ganapathy V (2004). Functional identification of SLC5A8, a tumor suppressor down-regulated in colon cancer, as a Na+-coupled transporter for short-chain fatty acids. J Biol Chem 279(14):13293-13296 Cited In: Binder, 2010 [Ref. #39].

Monira S, Alam NH, Suau A, Magne F, Nair GB, Karmakar PC et al. (2009). Time course of bacterial diversity in stool samples of malnourished children with cholera receiving treatment. J Pediatr Gastroenterol Nutr 48(5):571-578.

Muir JG, Birkett A, Brown I, Jones G, O'Dea K (1995a). Food processing and maize variety affects amounts of starch escaping digestion in the small intestine. Am J Clin Nutr 61(1):82-89.

Muir JG, Lu ZX, Young GP, Cameron-Smith D, Collier GR, O'Dea K (1995b). Resistant starch in the diet increases breath hydrogen and serum acetate in human subjects. Am J Clin Nutr 61(4):792-799.

Muir JG, Walker KZ, Kaimakamis MA, Cameron MA, Govers MJ, Lu ZX et al. (1998). Modulation of fecal markers relevant to colon cancer risk: a high-starch Chinese diet did not generate expected beneficial changes relative to a Western-type diet. Am J Clin Nutr 68(2):372-379.

Muir JG, Yeow EG, Keogh J, Pizzey C, Bird AR, Sharpe K et al. (2004). Combining wheat bran with resistant starch has more beneficial effects on fecal indexes than does wheat bran alone. Am J Clin Nutr 79(6):1020-1028

Murphy MM, Douglass JS, Birkett A (2008). Resistant starch intakes in the United States. J Am Diet Assoc 108(1):67-78.

Newberne PN, Conner MW, Estes P (1988). The influence of food additives and related materials on lower bowel structure and function. Toxicol Pathol 16(2):184-197.

Noakes M, Clifton PM, Nestel PJ, Le Leu R, McIntosh G (1996). Effect of high-amylose starch and oat bran on metabolic variables and bowel function in subjects with hypertriglyceridemia. Am J Clin Nutr 64(6):944-951.

Nugent AP (2005). Health properties of resistant starch. Nutr Bull BNF 30:27-54.

Pal S, Murugan C, Clarke JM, Young GP, Ramakrishna BS, Lockett T (2013). Esterified starch as a treatment for acute gastroenteritis-a double blind randomized controlled trial. Gastroenterology 144(5):S-904 [abstract Tu2012].

Phillips J, Muir JG, Birkett A, Lu ZX, Jones GP, O'Dea K et al. (1995). Effect of resistant starch on fecal bulk and fermentation-dependent events in humans. Am J Clin Nutr 62(1):121-130.

Platel K, Shurpalekar KS (1994). Resistant starch content of Indian foods. Plant Foods Hum Nutr 45(1):91-95.

Raghupathy P, Ramakrishna BS, Oommen SP, Ahmed MS, Priyaa G, Dziura J et al. (2006). Amylase-resistant starch as adjunct to oral rehydration therapy in children with diarrhea. J Pediatr Gastroenterol Nutr 42(4):362-368.

Ramakrishna BS, Venkataraman S, Srinivasan P, Dash P, Young GP, Binder HJ (2000). Amylase-resistant starch plus oral rehydration solution for cholera. N Engl J Med 342(5):308-313.

Ramakrishna BS, Subramanian V, Mohan V, Sebastian BK, Young GP, Farthing MJ et al. (2008). A randomized controlled trial of glucose versus amylase resistant starch hypo-osmolar oral rehydration solution for adult acute dehydrating diarrhea. PLoS ONE 3(2):e1587. doi 1510.1371/journal.pone.0001587.

Rao SSC, Edwards CA, Austen CJ, Bruce C, Read NW (1988). Impaired colonic fermentation of carbohydrate after ampicillin. Gastroenterology 94:928-932.

Richardson PH, Jeffcoat R, Shi Y-C (2000). High amylose starches from biosynthesis to their use as food ingredients. MRS Bull (Dec.):20-24.

Roberts J, Jones GP, Rutishauser IHE, Birkett A, Gibbons C (2004). Resistant starch in the Australian diet. Nutr Diet 61(2):98-104.

Saito K, Ito T, Kuribayashi T, Mochida K, Nakakuki T, Shibata M et al. (2001). Effect of raw and heat-moisture treated high-amylose corn starch on fermentation by the rat cecal bacteria. Starke [Starch] 53(9):424-430.

Sajilata MG, Singhal RS, Kulkarni PR (2006). Resistant starch—a review. Compr Rev Food Sci Food Safety 5(1):1-17.

Sandle GI (1998). Salt and water absorption in the human colon: a modern appraisal. Gut 43(2):294-299.

Sandstedt RM, Strahan D, Ueda S, Abbot RC (1962). The digestibility of high-amylose corn starches compared to that of other starches. The apparent effect of the *ae* gene on susceptibility to amylase action. Cereal Chem 39:123-131.

Scheppach W (1994). Effects of short chain fatty acids on gut morphology and function. Gut 35(1, Suppl.):S35-S38.

Shen L, Keenan MJ, Raggio A, Williams C, Martin RJ (2011). Dietary-resistant starch improves maternal glycemic control in Goto-Kakizaki rat. Mol Nutr Food Res 55(10):1499-1508.

Slavin J (2013). Fiber and prebiotics: mechanisms and health benefits. Nutrients 5(4):1417-1435.

Slizewska K, Kapusniak J, Barczynska R, Jochym K (2012). Resistant dextrins as prebiotic. In: Chang C-F, editor. *Carbohydrates - Comprehensive Studies on Glycobiology and Glycotechnology*. Rijeka, Croatia: InTech Europe, pp. 261-288. Available at: http://cdn.intechopen.com/pdfs-wm/41117.pdf.

Subramanya S, Ramakrishna BS, Binder HJ, Farthing MJ, Young GP (2006). Evaluation of oral rehydration solution by whole-gut perfusion in rats: effect of osmolarity, sodium concentration and resistant starch. J Pediatr Gastroenterol Nutr 43(5):568-575.

Topping DL, Clifton PM (2001). Short-chain fatty acids and human colonic function: Roles of resistant starch and nonstarch polysaccharides. Physiol Rev 81(3):1031-1064.

Topping DL, Gooden JM, Brown IL, Biebrick DA, McGrath L, Trimble RP et al. (1997). A high amylose (amylomaize) starch raises proximal large bowel starch and increases colon length in pigs. J Nutr 127(4):615-622.

Topping DL, Fukushima M, Bird AR (2003). Resistant starch as a prebiotic and synbiotic: state of the art. Proc Nutr Soc 62(1):171-176.

Topping DL, Bajka BH, Bird AR, Clarke JM, Cobiac L, Conlon MA et al. (2008). Resistant starches as a vehicle for delivering health benefits to the human large bowel. Microb Ecol Health Dis 20(2):103-108.

U.S. EPA (1984). *Starch Manufacturing: A Profile: Final Report*. (EPA Contract no 68-D1-0143, RTI Project no 35U-5681-71 DR). Prepared by Research Triangle Park (NC): Center for Economics Research, Research Triangle Institute (RTI) for Research Triangle Park (NC): U.S. Environmental Protection Agency (U.S. EPA), Office of Air Quality Planning and Standards. Available at: http://www.epa.gov/ttnecas1/regdata/IPs/Starch%20Manufacturing_IP.pdf.

U.S. FDA (1985). Unmodified food starches and acid-modified starches; Proposed affirmation of GRAS status as direct and indirect human food additives; Proposed rule [21 CFR Parts 182, 184 and 186); Docket No. 84N-0341]. Fed Regist (US) 50(62):12821-12825.

U.S. FDA (1993). Appendix I. Table 14. Conversion table for test chemical treatment doses used in PAFA. In: *Priority Based Assessment of Food Additives (PAFA) Database*. Washington (DC): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN), p. 58. Available at: http://www.fda.gov/ohrms/DOCKETS/DOCKETS/95s0316/95s-0316-Rpt0272-36-Appendix-D-Reference-F-FDA-vol205.pdf.

U.S. FDA (1996). Regulation of medical foods: Advance notice of proposed rulemaking (21 CFR Ch. I. [Docket No. 96N–0364] RIN 0905–AD91). Fed Regist (US) 61(231):60661-60671.

U.S. FDA (2004). Withdrawal of certain proposed rules and other proposed actions [21 CFR Chapter 1; Docket No. 2002N-0434]. Fed Regist (US) 69(227):68831-68838.

U.S. FDA (2006). Chapter 21 – Food composition, standards, labeling and economics. Medical Foods Program – Import and domestic (FY 06/07/08). In: *Compliance Program Guidance Manual*. (Program 7321.002). College Park (MD): U.S. Food and Drug Administration (U.S. FDA). Available at: http://www.fda.gov/downloads/Food/ComplianceEnforcement/UCM073339.pdf.

U.S. FDA (2011). *The Straight Poop on Kids and Diarrhea*. (FDA Consumer Heal t h Information, Oct. 2011). College Park (MD): U.S. Food and Drug Administration (U.S. FDA). Available at: http://www.fda.gov/forconsumers/consumerupdates/ucm277359.htm [Page Last Updated: 11/15/2013].

U.S. FDA (2013a). *Guidance for Industry: Frequently Asked Questions About Medical Foods.* College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN). Available at: http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/MedicalFoods/ucm054048.htm [May 1997; May 2007; Revised August 2013].

U.S. FDA (2013b). Part 182—Substances generally recognized as safe. Section §182.90—Substances migrating to food from paper and paperboard products. In: *U.S. Code of Federal Regulations (CFR). Title 21: Food and Drugs* (Food and Drug Administration). Washington (DC): Food and Drug Administration (U.S. FDA), U.S. Government Printing Office (GPO). Available at: http://www.gpo.gov/fdsys/browse/collectionCfr.action?collectionCode=CFR.

U.S. FDA (2013c). Part 182—Substances generally recognized as safe. Section §182.70—Substances migrating from cotton and cotton fabrics used in dry food packaging. In: *U.S. Code of Federal Regulations (CFR). Title 21: Food and Drugs* (Food and Drug Administration). Washington (DC): Food and Drug Administration (U.S. FDA), U.S. Government Printing Office (GPO). Available at: http://www.gpo.gov/fdsys/browse/collectionCfr.action?collectionCode=CFR.

UNICEF (2003). *New formulation of Oral Rehydration Salts (ORS) with reduced osmolarity*. (Technical Bulletin no 9). New York (NY): United Nations Children's Fund (UNICEF). Available at: http://www.unicef.org/supply/files/Oral_Rehydration_Salts(ORS)_.pdf.

UNICEF/WHO (2009). *Diarrhoea: Why Children are Still Dying and What Can be Done*. New York (NY): United Nations Children's Fund (UNICEF) / Geneva, Switz.: World Health Organization (WHO). Available at: http://www.who.int/maternal_child_adolescent/documents/9789241598415/en/.

van den Berg J, Berger MY (2011). Guidelines on acute gastroenteritis in children: a critical appraisal of their quality and applicability in primary care. BMC Fam Pract 12:134. doi: 10.1186/1471-2296-12-134.

van Munster IP, Tangerman A, Nagengast FM (1994a). Effect of resistant starch on colonic fermentation, bile acid metabolism, and mucosal prolife. Dig Dis Sci 39(4):834-842.

van Munster IP, de Boer HM, Jansen MC, de Haan AF, Katan MB, van Amelsvoort JM et al. (1994b). Effect of resistant starch on breath-hydrogen and methane excretion in healthy volunteers. Am J Clin Nutr 59(3):626-630.

Vesikari T, Isolauri E, Baer M (1987). A comparative trial of rapid oral and intravenous rehydration in acute diarrhoea. Acta Paediatr Scand 76(2):300-305.

Victora CG, Bryce J, Fontaine O, Monasch R (2000). Reducing deaths from diarrhoea through oral rehydration therapy. Bull World Health Organ 78(10):1246-1255.

Wang Y-J, White P, Pollak L, Jane J (1993). Characterization of starch structures of 17 maize endosperm mutant genotypes with Oh43 inbred line background. Cereal Chem 70(2):171-179.

Wang X, Brown IL, Evans AJ, Conway PL (1999). The protective effects of high amylose maize (amylomaize) starch granules on the survival of *Bifidobacterium* spp. in the mouse intestinal tract. J Appl Microbiol 87(5):631-639.

WGO (2008). *Acute Diarrhea*. (Practice Guideline). Munich, Germany: World Gastroenterology Organisation (WGO). Available at: http://www.worldgastroenterology.org/assets/downloads/en/pdf/guidelines/01_acute_diarrhea.pdf.

WGO (2012). *Acute Diarrhea in Adults and Children: a Global Perspective*. (Official Guidelines). Munich, Germany: World Gastroenterology Organisation (WGO). Available at: http://www.worldgastroenterology.org/assets/export/userfiles/Acute%20Diarrhea_long_FINAL_120604.pdf.

White PJ (2001). Properties of corn starch. In: Hallauer AR, editor. *Specialty Corn, 2nd edition*. Boca Raton (FL): CRC Press LLC, pp. 32-62 [See pp. 51-52]. Available at: http://books.google.ca/books?id=jw1pkkTu8o4C&lpg=PA51&ots=Dwr2D-Mob4&dq=high%20amylose%20corn%20starch%20gelatinize&pg=PA51#v=onepage&q=high%20amylose%20corn%20starch%20gelatinize&f=false.

WHO (2002). New formula oral rehydration salts. In: *WHO Drug Information*. (WHO Drug Information Vol. 16, No. 2, 2002). Geneva, Switz.: World Health Organization (WHO), pp. 121-122. Available at: http://apps.who.int/medicinedocs/en/d/Js4950e/2.4.html.

WHO (2004). *First Steps for Managing an Outbreak of Acute Diarrhoea: Leaflet*. (WHO/CDS/CSR/NCS/2003.7 Rev.1). Geneva, Switz.: World Health Organization (WHO), Global Task Force on Cholera Control. Available at: http://www.who.int/cholera/publications/firststeps/en/.

WHO (2005). *The Treatment of Diarrhoea: a Manual for Physicians and Other Senior Health Workers*. (4th rev: WHO/CDD/SER/80.2). Geneva, Switz.: World Health Organization (WHO). Available at: http://www.who.int/maternal_child_adolescent/documents/9241593180/en/.

WHO (2013). *Diarrhoeal Disease*. (Fact Sheet no 330). Geneva, Switz.: World Health Organization (WHO). Available at: http://www.who.int/mediacentre/factsheets/fs330/en/.

WHO/ICDDRB (1995). *25 Years of ORS—Joint WHO/ICDDRB Consultative Meeting on ORS Formulation,* Dec. 10-12, 1994, Dhaka, Bangladesh. (CDR/CDD/95.2). Geneva, Switz.: World Health Organization (WHO), Division of Diarrhoeal and Acute Respiratory Disease Control / Dhaka, Bangladesh: International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDRB). Available at: http://apps.who.int/iris/bitstream/10665/61332/1/CDR_CDD_95.2.pdf?ua=1.

WHO/UNICEF (2006). *Oral Rehydration Salts: Production of the new ORS*. (WHO/FCH/CAH/06.1). Geneva, Switz.: World Health Organization (WHO) / Copenhagen, Denmark: United Nations Children's Fund (UNICEF) Supply Division. Available at: http://www.who.int/maternal_child_adolescent/documents/fch_cah_06_1/en/.

Young AH (1984). Fractionation of starch. In: Whistler RL, BeMiller JN, Paschell EF, editors. *Starch: Chemistry and Technology, 2nd edition*. New York (NY): Academic Press, pp. 249-284 [See p. 272]. Available at: http://books.google.ca/books?id=pvAzqk2pAIsC&lpg=PA252&ots=jm02jysJmy&dq=Young%20fractionation%20of%20starch&pg=PA272#v=onepage&q=Young%20fractionation%20of%20starch&f=false.

Expert Panel Consensus Statement Concerning the Generally Recognized as Safe (GRAS) Determination of High-Amylose Maize Starch for use in Oral Rehydration Solutions that are Medical Foods

May 25, 2015

Yale University (Yale) intends to market High-Amylose Maize Starch (HAMS) as an ingredient[1] in Oral Rehydration Solutions (ORS), defined as medical foods in accordance with section 5(b) of the Orphan Drug Act 21 U.S.C. 360ee(b)(3), at a use level of 50 g/L of the finished and/or reconstituted ready-to-drink product.

An Expert Panel of independent scientists, qualified by their scientific training and relevant national and international experience to evaluate the safety of food ingredients, was specially convened to conduct a critical and comprehensive evaluation of the available pertinent data and information, and determine whether the proposed use of HAMS as a nutrient source of low-osmolar glucose and fermentable carbohydrate in ORS is safe and suitable and would be Generally Recognized as Safe (GRAS) based on scientific procedures. The Expert Panel consisted of Dr Joseph F. Borzelleca, Ph.D. (Virginia Commonwealth University School of Medicine); Dr. George C. Fahey Jr, Ph.D. (University of Illinois), and Dr. John Doull, M.D., Ph.D. (University of Kansas Medical Center). In addition to the Expert Panel, an advisory panel of experts in ORS also participated in the meeting and served as support to the Expert Panel providing advice on matters related to colonic ion transport and ORS use for rehydration of diarrhea. The advisory panel consisted of the following experts: Dr. Henry J. Binder MD (Yale School of Medicine, New Haven, CT); Graeme P. Young MD (Flinders University, Adelaide, Australia) and Dr. Ian Brown Ph.D. (Flinders University, Adelaide, Australia).

The Expert Panel, independently and collectively, critically evaluated a dossier[2] which included details pertaining to the method of manufacture and product specifications, supporting analytical data, proposed use in ORS, estimated exposure under the proposed use, and a comprehensive assessment of the available scientific literature pertaining to the safety of the proposed use of HAMS. The Expert Panel also evaluated other information deemed appropriate or necessary. Following its independent, critical evaluation of such data and information, the Expert Panel met *via* teleconference on August 18, 2014. The Expert Panel unanimously concluded that the proposed use described herein for HAMS, meeting appropriate food-grade specifications,and manufactured consistent with current Good Manufacturing Practice (cGMP), is safe and suitable and GRAS based on scientific procedures. A summary of the basis for the Expert Panel's conclusion is provided below.

[1] Nutrient - source of low-osmolar glucose and fermentable carbohydrates
[2] Documentation Supporting the Determination that High-Amylose Maize Starch (HAMS) is Generally Recognized as Safe (GRAS) for Use as an Ingredient in Oral Rehydration Solutions that are Medical Foods

SUMMARY AND BASIS FOR GRAS

The ingredient that is the subject of this GRAS evaluation is high-amylose maize starch, which meets the specifications for unmodified food starches as defined in the Food Chemical Codex (FCC, 2014). ORS are characterized as solutions of water, electrolytes, and a carbohydrate source (61 FR 60661 – U.S. FDA, 1996), and in the United States (U.S.), oral rehydration solutions are defined as a form of distinctive nutritional support for rehydration during episodes of diarrhea, and are regulated as medical foods (U.S. FDA, 2006). , A number of ORS formulations are currently marketed in the U.S. (*e.g.,* Pedialyte®, Naturalyte®, Enfalyte®, and CeraLyte®) that are intended to promote the replacement of water and salts lost during acute diarrhea in children and adults (U.S. FDA, 2011). There is no prior history of HAMS or other types of resistant starches being used as a carbohydrate source in ORS in the U.S., though rice-derived ORS containing carbohydrate polymers (*e.g.,* Ricelyte®, CeraLyte®) are currently marketed.

High-amylose maize starch is intended for use as a nutrient source of low-osmolar glucose and fermentable carbohydrates for addition to ORS formulations that are currently marketed in the U.S. for maintaining water and electrolyte balance during episodes of viral or bacterial gastroenteritis among the general population. High-amylose maize starch will be added to ORS formulations at a use level of 50 g/L in the finished reconstituted product. The recommended conditions of use of ORS products containing HAMS will be consistent with current practices for the management of acute diarrhea endorsed by the World Health Organization (WHO), the World Gastroenterology Organisation (WGO), Centers for Disease Control and Prevention[3] (CDC) (CDC, 2003; WHO, 2005; WGO, 2012). According to these guidelines, oral rehydration therapy typically consists of 2 phases, rehydration and maintenance. In individuals exhibiting at least some signs of dehydration, there is an initial rehydration phase where large amounts of ORS are consumed (usually within the first 3 to 4 hours of dehydration) in order to replace the existing fluid and electrolyte loss (CDC, 2003; WHO, 2005; WGO, 2012). This is followed by a maintenance phase where ORS are consumed to replace ongoing losses during subsequent episodes of diarrhea (and possibly vomiting) (CDC, 2003; WHO, 2005; WGO, 2012). Based on the recommended volumes of ORS that should be consumed within the first 24 hours of diarrhea-associated dehydration, a use level of 50 g/L of HAMS in ORS could result in a theoretical maximum intake of 105 to 250 g HAMS among children (>2 years) and teenagers (up to 15 years), and up to 300 g HAMS among adults. The Expert Panel recognized that these maximum estimates are more likely to reflect extreme situations in underdeveloped nations, where cholera diarrhea (which is associated with severe fluid and electrolytes loss) is prevalent (WHO, 2005; UNICEF/WHO, 2009). Among healthy subjects in the developed world, where acute diarrhea is most often caused by viral infections (*e.g.*, rotavirus or human caliciviruses),

[3] The American Academy of Pediatrics has also indicated that they accept and endorse the guidelines for managing acute gastroenteritis that were published by the CDC (AAP, 2004).

the intake of ORS is expected to be less (Vesikari *et al*., 1987; Elliott *et al*., 1989; Guiraldes *et al*., 1995). For example, Vesikari *et al*. (1987) evaluated the use of rapid rehydration therapy in acute diarrhea in 37 Finnish children under the age of 5 hospitalized for acute diarrhea and dehydration. Subjects were provided oral or intravenous rehydration during 6 to 12 hours, and total fluid intake at 6 hours among ORS users during the trial ranged from 152 to 368 mL (95% CI). Dietary consumption of HAMS from this level of ORS use would equate to 7.6 to 18.4 g HAMS per child. It is also important to note that the largest amount of ORS will be consumed only within the first 24 hours of intervention by individuals with mild to moderate dehydration, as large amounts are initially required to replenish the existing fluid and electrolyte losses. During the subsequent maintenance phase, it is recommended that children (age 2 to 10 years) consume approximately 1 L/day (providing 50 g/day of HAMS), and adults consume approximately 2 L/day (providing 100 g/day of HAMS), to replace ongoing fluid losses from each loose stool or vomiting episode (WHO, 2004, 2005). Moreover, exposures to HAMS from its proposed uses in ORS will be limited to short-term consumption since acute diarrhea typically lasts less than 7 days, and not longer than 14 days (Guarino *et al.*, 2008). As HAMS and other resistant starches are widely present in the diet, the potential for additive dietary exposure from the background diet was considered; however, the Expert Panel considered potential dietary exposures from foods containing HAMS to be trivial relative to intakes from ORS uses. Since gastroenteritis is typically associated with reduced feeding, additive consumption of resistant starches from the background diet would likely be minimal during ORS consumption.

Maize starch is a common source of dietary macronutrients (*e.g*., carbohydrates) with a long history of consumption among populations globally. Starch is a glucose homopolymer that typically exists in two forms in maize cultivars and other plant varieties (*e.g*., wheat, and potato) as amyolse and amylopectin (Morita *et al*., 2007). Amylose is a linear structure comprised on glucose units lined *via alpha*-(1,4) glycosidic bonds. Amylopectin is a branched molecule containing *alpha*-(1,4) linked glucose units with *alpha*-(1,6) branching points. The compact helical structure of the amylose molecules produce a conformation that is more difficult to digest by pancreatic amylases relative to the open structure of amylopectin (Morita et al., 2007). Amylose also is naturally present as inclusion complexes with lipids, producing granules that further resist digestion in small intestine. Naturally occurring variants of corn that contain a high-amylose starch content (*i.e.*, amylomaize) were first produced in the 1940's through conventional breeding programs, and these hybrids became commercially available in 1958 (BeMiller, 2009). Amylomaize grains typically contain 55 to >90% amylose starch depending on the particular cultivar from which the starch is derived. This compares to starch derived from conventional low-amylose maize cultivars of corn or wheat which typically contain amylose contents below 20 to 30% (Wang *et al.*, 1993; Richardson *et al.*, 2000). In 1979, the Select Committee on GRAS Substances (SCOGS) evaluated the safety of various types of starches, including HAMS. It was concluded that "*there is no evidence in the available information on unmodified or pregelatinized corn, high amylose corn, waxy maize, wheat, milo (also called grain sorghum starch), rice, potato, tapioca or arrowroot starch that demonstrates or suggests*

reasonable grounds to suspect a hazard to the public when they are used at levels that are now current or that might reasonably be expected in the future" (FASEB, 1979). On this basis, the U.S. Food and Drug Administration drafted a proposed rule (50 FR 12821) to amend Part 184 (Direct Food Substances Affirmed as Generally Recognized as Safe) of Title 21 of the Code of Federal Regulations to include unmodified food starches (21 CFR §184.1847). Under this proposed rule, unmodified food starches, including high-amylose corn starch, are considered GRAS for use in foods with no limitations other than cGMP (50 FR 12821)[4].

In many other jurisdictions worldwide, HAMS is also permitted for general food use *quantum satis*. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) stated that native food starches, amylose and amylopectin, should be considered as foods/food ingredients rather than food additives (JECFA, 1969, 1974). The acceptable daily intake level was determined as "not specified", a designation that is given to ingredients that are of very low toxicity and that JECFA do not consider to pose a hazard to human health at the total dietary intake of the substance arising from its use at the levels necessary to achieve the desired effect and from its background intake in foods (JECFA, 1974, 1982).

High-amylose maize starch is a type of resistant starch (RS_2) as its digestion within the small intestine is incomplete, and a fraction of the ingested dose will reach the large intestine intact (Topping and Clifton, 2001). Using consumption data from the National Health and Nutrition Examination Survey 1999-2002, and published literature values of resistant starch content of specific foods, Murphy *et al.* (2008) estimated the average dietary consumption of resistant starch by the total U.S. population (≥1 year old) to be 4.9 g/day (range of 2.8 to 7.9 g/day). The Institute of Medicine (IOM) of the National Academy of Sciences has also estimated the median dietary intake of total fiber[5], including naturally occurring resistant starches such as HAMS, to range from 16.5 to 17.9 g/day for men and 12.1 to 13.8 g/day for women in the United States (IOM, 2005). By comparison, the Adequate Intake[6] levels for total fiber established by the IOM, which are based mainly on the beneficial health effects of consuming fiber (such as the reduced risk of coronary heart disease), are 19 g/day for children (1 to 3 years), 25 g/day for children (4 to 8 years), 21 to 26 g/day for females (9 years and older), and 30 to 38 g/day for males (9 years and older) (IOM, 2005).

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[4] This proposed rule, along with several other proposed GRAS actions listed in the Notice of Intent, were ultimately withdrawn due to the large backlog of pending proposals and limited resources of the FDA to adequately review the comments and take action in a timely manner (69 FR 68831). Nevertheless, the FDA has indicated that withdrawal of these proposed rules does not affect the regulatory status of the ingredients listed in these documents (69 FR 68831).

[5] The IOM considers resistant starch that is naturally occurring (*e.g.*, HAMS) or created during normal processing of a food to be classified as "dietary fiber", while isolated or extracted non-digestible carbohydrates (*e.g.*, using chemical, enzymatic, or aqueous steps) are considered as "functional fiber" (IOM, 2005). Total fiber is the sum of "dietary fiber" plus "functional fiber" (IOM, 2005).

[6] The IOM defines Adequate Intake as "*the recommended average daily intake level based on observed or experimentally determined approximations or estimates of nutrient intake by a group (or groups) of apparently healthy people that are assumed to be adequate*" (IOM, 2005).

In general, starch digestion is mediated mainly through the actions of pancreatic α-amylase in the small intestine, which catalyzes the hydrolysis of the α-D-(1,4)-glycosidic bonds to smaller oligosaccharides that are then further digested into free glucose units by membrane-bound α-glucosidases and other amylolytic enzymes (Filer *et al.*, 1988; Topping and Clifton, 2001; de Sales *et al.*, 2012). Pancreatic amylase activity may be reduced during the the first few months of life (McClean and Weaver, 1993), therefore the Expert Panel sought advice from the ORS advisory panel on the use of HAMS in ORS by infants of all ages. It was concluded that there is currently no substantive scientific evidence to suggest starch digestion in young infants is reduced in a quantitatively meaningful manner such that it would affect that the safe use of ORS in infants. This view is corroborated by the wide-spread and long-history of safe use of other starch based ORS products in infants, specifically, rice-based ORS, which are generally recognized as safe and suitable for rehydration in infants of all ages under all conditions of gastroenteritis. . The *in vitro* digestibility of HAMS has been reported at 30 to 55% following incubation with α-amylase (Sandstedt *et al.*, 1962; Fujita *et al.*, 1989; Liu *et al.*, 1997); however, greater digestion efficiencies are observed *in vivo*. For example, the ileal digestibility of HAMS in pigs, which are known to have similar digestive processes to humans, was reported at 87.8% following dietary administration of HAMS (85% amylose) at 51.5% w/w (Bird *et al.*, 2007) for 21 days. Among ileostomists (n=7) who consumed custards containing 20 g of cooked and cooled HAMS, the ileal digestibility of HAMS was reported at 72.9% (Clarke *et al.*, 2007). As food grade starches isolated from high amylose maize cultivars (*i.e.*, HAMS) can contain resistant starch contents between 55 to 90%, the Expert Panel considered the effects of using HAMS with different resistant starch contents in ORS. Morita and colleagues (2007), evaluated the *in vitro* and *in vivo* digestibility of four HAMS preparations, obtained from different maize cultivars, containing amylose contents of between 53.8 to 90.0% w/w. The authors reported that the amylose content of HAMS did not appreciably influence pancreatic alpha-amylase digestion of the starch granules. The authors also reported that the amylose content of HAMS did not affect starch digestibility when provided in the diet (62.5% w/w) of ileorectostomized rats over 7 days. The Expert Panel concluded that there are no meaningful differences in the *in vitro* or *in vivo* digestibility of HAMS with differing amylose content.

Resistant starches such as HAMS that escape digestion in the small intestine are transported to the colon where they are fermented by the indigenous microbiota to release gases (H_2, CH_4, and CO_2) and short-chain fatty acids (mainly acetate, propionate, and butyrate) (Brown, 1994; van Munster *et al.*, 1994a; Phillips *et al.*, 1995; Topping and Clifton, 2001). The fermentative effect of HAMS ingestion towards increasing the levels of total short-chain fatty acids (SCFA) and lowering pH in the lower gastrointestinal tract has been well demonstrated in animal and clinical studies (De Schrijver *et al.*, 1999; Kasaoka *et al.*, 1999a,b; Ferguson *et al.*, 2000; Saito *et al.*, 2001; Topping and Clifton, 2001; Le Leu *et al.*, 2003; Nugent, 2005; Bird *et al.*, 2007; Murphy *et al.*, 2008). The fermentation of resistant starches is expected to be generally

complete, even following consumption of large dietary quantities (FAO/WHO, 1998; Bird *et al.*, 2010). For example, studies conducted by Ramakrishna and colleagues in a group of 15 healthy subjects administered a warm flavored drink containing 20 or 40 g of HAMS reported a total gastrointestinal tract digestibility of between 97 to 99% (Ramakrishna, 2012). Studies in pigs administered HAMS in the diet at 51.5% w/w for 21 days demonstrated that HAMS had a colonic digestibility of 93.5%, and therefore the large intestine, under normal conditions, has a high capacity to ferment large quantities of resistant starch that escape digestion in the small intestine. The colon also has a high capacity for absorption of SCFA, and the majority (95 to 99%) of the SCFA produced by microbial fermentation is rapidly absorbed in the colon *via* non-ionic diffusion and/or by transporter mechanisms that are linked to sodium absorption (Scheppach, 1994; Hijova and Chmelarova, 2007; Binder, 2010).

Traditional animal toxicology studies characterizing the sub-chronic or long-term consumption of HAMS or other naturally occurring high-amylose starch products (*i.e.*, potato starch, green banana starch) were not identified in the comprehensive search of the literature; however, the Expert Panel noted that resistant starch is a widely consumed and common dietary macronutrient and safety concerns related to the consumption of large quantities of HAMS in ORS products would be limited to issues of acute intolerance, and suitability of HAMS as a nutrient source of glucose and fermentable carbohydrates for use in ORS. In short-term studies (≤4 weeks), administration of HAMS in the diet of pigs at high dietary levels of 51.5% [20.6 g/kg body weight/day (FAO/WHO, 2009)] and up to 40% in the diet [40 g/kg body weight/day (U.S. FDA, 1993)] of rats was generally well-tolerated without evidence of diarrhea. Reduced growth reported in some studies (*e.g.*, Ferguson *et al.*, 2000; Bird *et al.*, 2007) was attributed to caloric dilution of the diet, as opposed to overt toxicity. Long-term consumption of resistant starch in the diet of rats was associated with cecal enlargement; however, this effect is commonly observed in the rodent following repeated ingestion of large amounts of fermentable carbohydrates and is an effect that is not relevant to the human situation (Newberne *et al.*, 1988).

The ingestion of large quantities of resistant starches is typically well tolerated in healthy individuals. van Munster (1994b) evaluated the consumption of 45 g of raw HAMS or maltodextrin in 19 healthy male subjects using a randomized placebo controlled cross-over design. Each dietary treatment was consumed for one week. No differences in gastrointestinal symptom scores (*i.e.*, bloating, flatulence, cramps, belching, diarrhea) were reported between treatment intervals at the end of the study. Similar findings were reported by Phillips *et al.*, (1995) in a group of 11 healthy volunteers (35.5±3.2 yrs; 5♀; 6♂) consuming a low resistant or high resistant starch diet (HAMS + green banana flour) for 3 weeks in a randomized cross-over fashion. Daily consumption of between 26 to 50 g of resistant starch per day was well-tolerated and effect of resistant starch diet was limited to statistically significant increases in flatulence and ease of defecation. No differences in abdominal distension, cramps, or diarrhea were reported between diet periods. In a systematic review, Grabitske and Slavin (2009) evaluated

the gastrointestinal effects of resistant starch and estimated the acceptable daily intakes of resistant starches (RS_2 and RS_3) from various sources to be approximately 45 g/day, based on the increased incidence of excessive flatulence reported at higher doses. Compared to other non-glycemic carbohydrates, resistant starches have a high laxation threshold, with only rare cases of diarrhea reported even at doses as high as 80 g/day (Grabitske and Slavin, 2009).

Under normal circumstances in healthy adults, approximately 8 to 9 L of fluids are absorbed daily from the gastrointestinal tract, which represents approximately 1.5 L of ingested fluids and approximately 7 L of various gastrointestinal secretions (*e.g.,* pancreatic juices, biliary juices, saliva) (Hall, 2011). The absorption of water in the small intestines and colon occurs strictly *via* passive diffusion, as substances (*e.g.*, electrolytes and nutrients such as glucose and amino acids) are actively transported across the intestinal epithelium, thereby generating an osmotic gradient (Hall, 2011). In cases of acute gastroenteritis, which is most often caused by infectious bacteria or viruses, diarrhea is induced by enterotoxins that disrupt the transport processes within the enterocytes (Kopic and Geibel, 2010; Binder *et al.*, 2014). The specific receptor and downstream signaling pathways affected is dependent on the specific enterotoxin; nevertheless, the majority of toxins ultimately increases the synthesis of intracellular cyclic adenosine monophosphate (cAMP) or cGMP within enterocytes/colonocytes, which are negative regulators of the Na-H/Cl-HCO_3 co-transporter (NHE3), thereby leading to the decreased absorption of sodium and chloride (Kopic and Geibel, 2010). Furthermore, the apical chloride channel is also affected, which leads to increased chloride secretion (Kopic and Geibel, 2010). Together, these changes result in increased osmotic pressure within the intestinal lumen, resulting in the loss of large amounts of water (Kopic and Geibel, 2010).

Oral rehydration solutions work primarily by restoring the electrolyte balance through glucose-stimulated sodium absorption from the small intestine, which in turn enhances water absorption from the osmotic gradient generated (Binder and Ramakrishna, 2004; Kelly and Nadeau, 2004). The action of the glucose-sodium transporter is not altered by the increase in intracellular cyclic nucleotides that are mediated by enterotoxins; therefore, glucose-based ORS are effective in stimulating electrolyte and fluid absorption even during infectious gastroenteritis (Binder *et al.*, 2014). Inclusion of food starches (*e.g.*, HAMS) as a source of glucose in ORS would provide a continuous supply of glucose as they are readily digested within the intestinal lumen, thereby promoting the absorption of electrolytes and fluid without increasing the osmotic load (Kelly and Nadeau, 2004; Gregorio *et al.*, 2009). Additionally, ORS containing resistant starches such as HAMS would also promote electrolyte and water absorption in a cAMP-independent manner in the colon, as a result of SCFA production from microbial fermentation, which stimulates sodium absorption *via* action of the SCFA-HCO_3 exchanger (Binder and Ramakrishna, 2004; Binder, 2010; Binder *et al.*, 2014).

In certain individuals with extensive fluid loss as a result of acute diarrhea, it is possible that large amounts of ORS may need to be consumed in order to manage the resulting diarrhea. At

use levels of 50 g/L in ORS, the maximum theoretical intakes of HAMS during the first 24 hours of ORS intake may be as high as 300 g among adults, which is considerably higher than the typical level of intake of resistant starches from the diet (*i.e.*, approximately 4.9 g/day) (Murphy *et al.*, 2008), or the maximum amount of resistant starch that has been shown to be well-tolerated in clinical studies (*i.e.*, 80 g/day) (Grabitske and Slavin, 2009). The ingestion of large doses of resistant starches that exceed the capacity for fermentation and/or SCFA absorption in the colon could potentially result in osmotic diarrhea; however, the capacity for SCFA absorption in humans is large. For example, Holtug *et al.* (1992) estimated the capacity for SCFA absorption in humans to be in the range of 550 to 1,150 mmol/day. Assuming that 75 mmol of SCFA could be produced from 10 g of fermentable carbohydrate (Cummings *et al.*, 1989), the maximum theoretical intake of HAMS from its intended uses in ORS (*i.e.*, 300 g within the first 24 hours of oral rehydration therapy) would result in the production of approximately 900 mmol of SCFA [300 g x 0.4 x 75 mmol SCFA/ 10 g] [7]. Furthermore, unlike malabsorbed lactose or other similar simple sugars which could cause osmotic diarrhea when consumed in large amounts, ingestion of large amounts of resistant starch, for which there is a high capacity for microbial fermentation in the colon, does not appear to pose any adverse gastrointestinal effects among well-nourished individuals living in regions with good personal hygiene (Annison and Topping, 1994; Topping and Clifton, 2001). The Expert Panel noted that potential effects on gastrointestinal intolerance following consumption of large dietary levels of resistant starch by healthy humans is not representative of acute gastroenteritis, where gastric emptying rates and fecal volume are increased, and where changes in the microbiota within the gastrointestinal tract are typically observed . Accordingly, the safety and suitability of HAMS-based ORS should be largely based on findings from well-designed studies in adults and children evaluating the capacity of HAMS based ORS to achieve adequate rehydration in cholera and non-cholera (*i.e.*, rotavirus) diarrhea.

The Expert Panel reviewed findings from four published studies evaluating the use of HAMS based ORS in adults and children with acute diarrhea. The use of HAMS for rehydration during cholera diarrhea was first evaluated in 2000 by Ramakrishna and colleagues. Forty-eight adolescent and adult subjects (14 to 58 years) admitted to the Christian Medical College and Hospital in Vellore India were randomized to 1 of 3 treatment groups receiving the following ORS therapy: 1) standard WHO ORS glucose-based formula (n=16; $\bar{x}$ = 36.4 yrs; 12♂/4♀); 2) 50 g/L Rice-flour based ORS (n=16; $\bar{x}$ = 37.4 yrs; 10♂/6♀); or 3) WHO glucose based ORS + 50 g/L HAMS (n=16; $\bar{x}$ = 33.9; 11♂/5♀). The osmolarity of all 3 solutions was similar at ~327 mOsm/kg. All subjects were confirmed as *Vibrio cholera* positive. The use of HAMS based ORS provided effective hydration as significant improvements in time to first formed stool and fecal weight were observed in the subjects randomized to the HAMS group. The authors also measured cumulative fecal recovery of starch in a subset of subjects provided 1 L of ORS providing 50 g of HAMS or 2 g of polyethylene glycol (PEG). At 12 hours recovery of PEG was

[7] Conservative assumption that 40% of ingested HAMS reaches the colon

95% complete, and in the subjects provided HAMS ORS 8.3±8.9 g of starch was recovered, demonstrating that the fermentation capacity of the large intestine was largely unaffected by the diarrhea.

Raghupathy *et al*. (2006) evaluated the use of HAMS as an adjunct to glucose based ORS in 183 children with mild to moderate diarrhea presenting to the Christian Medical College and Hospital in Vellore India. Children were randomized to receive standard WHO glucose based ORS (n=91; $\bar{x}$ = 11.5±0.6 mo; 8.2±0.2 kg body weight; glucose = 90 mmol); or standard WHO glucose based ORS + 50 g/L of HAMS (n=87; $\bar{x}$ = 12.0±0.6 mo; 8.2±0.2 kg body weight). Twenty-eight subjects in the HAMS-ORS and 30 subjects in the control ORS group were rotavirus positive. Total ORS consumed (95% CI) in the study ranged between 67.1 to 92.1 mL in the HAMS-ORS group and between 74.1 to 100.4 mL in the control ORS group. The use of HAMS as an adjunct to ORS provided effective rehydration in the subjects as time from enrollment to last formed stool was reduced by ~50% in the HAMS group. Trends towards reduced stool output in the first 24 hours and total diarrheal stool output also were reported. These findings are suggestive of increased rehydration from colonic fermentation. Subgroup analyses of children with rotavirus did not reveal differences between the groups in stool volume. No differences between the groups in unscheduled intravenous fluids or diarrhea persisting beyond 72 hours were reported. A limitation of this study was the use of standard WHO glucose based ORS, which has now largely been replaced with low-osmolar glucose solution. However, there were no findings in this study to suggest that use of HAMS in ORS may be either ineffective as a rehydration nutrient and/or unsafe.

Alam *et al.* (2009) investigated the safety of rapid intravenous rehydration and three different ORS formulations in 175 severely malnourished children with cholera. The trial was conducted at the Dhaka hospital of the International Centre for Diarrhoeal Disease Research Bangladesh. Participants were randomized to 1 of 3 study groups assigned to receive standard glucose-based ORS (n = 58; $\bar{x}$ = 27.17±12.36 mo; 26♂32♀; 6.90±1.32 kg body weight); glucose-ORS + 50 g/L HAMS (n=59; $\bar{x}$ = 28.36±13.42 mo; 34♂/25♀; 7.09±1.52 kg body weight); or 50 g/L rice-based ORS (n = 58; $\bar{x}$ = 27.33±11.97 mo; 32♂/26♀; 6.78±1.43 kg body weight). The electrolyte composition of the ORS formulations were modified slightly from the standard WHO formulation, however, concentrations were equivalent between groups. Intravenous fluid was provided to 149 children (95% CI = 96 to 109 mL/kg), and all were rehydrated in 6 hours. All children without apparent extra-intestinal infection received ampicillin and gentamicin for 5 days. All subjects received erythromycin for cholera every 6 hours for 3 days. Body weights and stool weight were measured throughout the study and vital signs, dehydration and signs of over-hydration were monitored every 6 hours. Cumulative intake of ORS over 72 hours was between 400 to 700 mL per kg body weight. All 3 treatments were effective in correcting dehydration. No significant differences in outputs of stool, vomit, or urine were reported between groups randomized to glucose ORS or glucose ORS + HAMS. Statistically significant reductions in stool output were reported in subjects receiving rice-based ORS. The duration of diarrhea and

number of children receiving unscheduled intravenous therapy did not differ between groups. The authors also reported that over the duration of ORS consumption, none of the participants developed features of overhydration or cardiac failure, hypoglycemia, severe hypo- or hyper-kalemia, or severe hypo- or hyper-natremia. Findings from this study address an important consideration pertaining to the ability of severely malnourished children to digest complex carbohydrates due to a possible lack of amylase, a finding that was not observed in the rice-ORS group. The study also demonstrates that antibiotic use during rehydration therapy does not impair the effectiveness of resistant starch as an ORS.

Ramakrishna *et al.* (2008) conducted a randomized, controlled clinical trial where 50 adult males with cholera or cholera-like diarrhea (<3 days duration) and moderate to severe dehydration were administered either WHO-recommended hypo-osmolar ORS (245 mOsm/kg) containing 13.5 g/L glucose (HO-ORS), or the same ORS formulation but with the glucose substituted with 50 g/L of HAMS (National Starch, USA). The age (mean±SD) of the participants was 37.8±13.7 years in the HAMS-ORS group and 42.6±12.0 years in the HAMS-ORS group, and 22 of the participants tested positive for *Vibrio cholerae*. All subjects received 300 mg of doxycyline as a single oral dose. The ORS was administered at 200 mL/hour, and 200 mL was given after each loose stool, until the first formed stool. The participants were hospitalized for 48 hours, or until stool consistency was reported as “normal”. The mean volume of ORS consumed over 48 hours was reported at 10 L (range 6.2 to 13.1 L) in the HO-ORS group, and at 7.2 L (range 5.7 to 10 L) in the HAMS-ORS group (not statistically significant). All subjects completed the study, and there were no significant differences in the incidence of hyponatremia, or serum levels of K^+ and creatinine in subjects receiving HAMS-ORS compared to HO-ORS. None of the subjects required dialysis for renal failure, and there was no significant difference in the need for unscheduled intravenous fluid therapy between groups. The diarrhea did not persist longer than 48 hours, except in one subject in the control glucose-based ORS group. Subjects administered the HAMS-ORS also had significantly shorter time to form first stool (*i.e.*, faster recovery from diarrhea), and significantly lower fecal weight from 12 to 24 hours and from 24 to 48 hours compared to subjects administered HO-ORS, an effect that is consistent with the additional water absorption attributed to colonic fermentation and absorption of SCFA. The Expert Panel considered this study to be well designed, utilized an appropriate comparator (*i.e*., low-osmolar glucose ORS), and included critical safety-related outcomes including the incidence of unscheduled intravenous rehydration and measures of hyper/hypo-natremia.

Preliminary findings from a recent randomized, double-blind, controlled study provide additional corroborative information that HAMS-based ORS are safe for use in ORS. The study was conducted using 100 adults with acute diarrhea (<3 days duration) and moderate or severe dehydration. Subjects were randomly allocated to receive reduced osmolarity glucose-ORS containing either 50 g/L of acetylated HAMS (n=49) or 50 g/L HAMS (n=51). Twenty-four of the subjects tested positive for *Vibrio cholerae* (12 per group). No adverse events were reported in

either group, as indicated by the absence of hyponatremia. The need for unscheduled intravenous fluid therapy was high in both groups as most subjects had pre-renal failure. Administration of ORS-containing acetylated HAMS (HAMSA) did not exacerbate the diarrhea; compared to subjects receiving HAMS-ORS, those who received HAMSA-ORS had a significantly shorter duration of diarrhea (median 24.5 hours *vs*. 36.5 hours), and no significant differences between groups were reported in the fecal weight collected from enrollment until the first formed stool.

From the findings reported from multiple randomized controlled studies in children and adults, one may conclude that the use of HAMS in ORS at a use level of 50 g/L would provide a safe and suitable source of low-osmolar glucose to the small intestine for facilitation of rehydration in all types of gastroenteritis diarrhea. The applicability of findings conducted in Indian populations to the American population was considered. Factor's typically resulting in potential variability between populations such as non-linear pharmacokinetics, a steep dose-response, genetic polymorphisms affecting metabolism, high potential for protein binding, or potential for dependence/abuse are not relevant to the situation in which resistant starch is used in ORS (ICH, 1998). The Expert Panel considered the intended use to be ethnically insensitive and findings from studies conducted in the Indian population would be relevant to the safety assessment. The Expert Panel further noted that historical experiences gained during the development of modern ORS formulations, which were based on large-scale studies evaluating the provision of ORS formulations to refugees from Bangladesh form the basis for the current global standard of care for the management of acute gastroenteritis, and have proven to be applicable to populations in developed countries (CDC, 1992, 2003).

The Expert Panel noted that rotavirus infection can result in disruption of membrane bound disaccharidases affecting carbohydrate metabolism; however, digestion of glucose polymers is predominantly mediated by glucoamylase and maltase, enzymes that are highly resistant to damage of the intestinal mucosa (Bentley *et al.*, 2001). Hypernatremic dehydration is a rare and serious situation that is unique to rotavirus infection and can occur in well-nourished children (Kaiser *et al*., 2012; Farthing *et al.*, 2013). Case-reports of worsening dehydration and hypernatremia have been reported in association with a maltodextrin based ORS preparation in Sweden in 2009. It was hypothesized that the administration of large quantities of glucose polymers could result in hypertonic dehydration if glucose absorption is impaired during rotavirus infection. A cause-effect relationship between the use of maltodexrin as a glucose source in ORS and rare incidences of hypernatremia was never established (Lidefelt *et al*., 2010), and the Expert Panel noted that HMAS being a low-osmotic and slowly digestible glucose source would be less likely to adversely affect intraluminal oslolarity in instances of sugar malabsorption than rapidly digestible glucose polymers such as maltodextrin. For example, incubation of HAMS with pancreatic amylases at 37ºC for 30 minutes resulted in a starch digestibility of ≤6% (Morita et al., 2007). Reduction of the risk of hypernatremia in children with gastroenteritis is most effectively addressed by the use of early rehydration

therapy with low sodium concentrations (60 mmol/L) (ESPGHAN, 1992). Multiple controlled studies of HAMS-based use in children and adults with cholera and non-cholera diarrhea have not identified evidence of hypernatremia, which is consistent with the long-history of safe use of other resistant starch based ORS formulations (*i.e*., rice-based ORS) for rehydration during all types of gastroenteritis (Gregorio *et al.*, 2009). The Expert Panel also noted that glucose polymer based ORS formulations have been marketed in the United States (*i.e*., ceralyte, ricelyte) for several years, and the Panel is not aware of publically available information to suggest that these products have been associated with adverse effects in children with gastroenteritis.

CONCLUSIONS

We, the members of the Expert Panel, have independently and collectively critically evaluated the information summarized above, and other data and information that we deemed pertinent to the safety of the proposed uses of High-Amylose Maize Starch. We unanimously conclude that High-Amylose Maize Starch, meeting appropriate food-grade specifications and manufactured in accordance with current Good Manufacturing Practice, is safe and suitable for use in Oral Rehydration Solutions defined as medical foods at a use level of 50 g/L.

We further unanimously conclude that High-Amylose Maize Starch, meeting appropriate food-grade specifications and manufactured in accordance with current Good Manufacturing Practice, is Generally Recognized as Safe based on scientific procedures, for use in Oral Rehydration Solutions defined as medical foods at a use level of 50 g/L.

It is our opinion that other qualified experts would concur with these conclusions.

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REFERENCES

AAP (2004). Managing acute gastroenteritis among children: oral rehydration, maintenance, and nutritional therapy (American Academy of Pediatrics). Pediatrics 114(2):507. Available at: http://pediatrics.aappublications.org/content/114/2/507.full.

Alam NH, Islam S, Sattar S, Monira S, Desjeux JF (2009). Safety of rapid intravenous rehydration and comparative efficacy of 3 oral rehydration solutions in the treatment of severely malnourished children with dehydrating cholera. J Pediatr Gastroenterol Nutr 48(3):318-327.

Annison G, Topping DL (1994). Nutritional role of resistant starch: Chemical structure vs physiological function. Annu Rev Nutr 14:297-320.

BeMiller JN (2009). One hundred years of commercial food carbohydrates in the United States. J Agric Food Chem 57(18):8125-8129.

Bentley D, Lifschitz C, Lawson M (2001). Acute and chronic diarrhea. In: *Pediatric Gastroenterology and Clinical Nutrition.* London (UK) / Chicago (IL): ReMedica Publishing. Available at: http://webcache.googleusercontent.com/search?q=cache:MFwO6mmm8yoJ:www.naspghan.org/wmspage.cfm%3Fparm1%3D126+&cd=1&hl=en&ct=clnk&gl=ca.

Binder HJ (2010). Role of colonic short-chain fatty acid transport in diarrhea. Annu Rev Physiol 72:297-313.

Binder HJ, Ramakrishna BS (2004). Resistant starch-An adjunct to oral rehydration solution: Not yet ready for prime time. J Pediatr Gastroenterol Nutr 39(4):325-327.

Binder HJ, Brown I, Ramakrishna BS, Young GP (2014). Oral rehydration therapy in the second decade of the twenty-first century. Curr Gastroenterol Rep 16(3):376 [8 p.]. DOI 10.1007/s11894-014-0376-2.

Bird AR, Conlon MA, Christophersen CT, Topping DL (2010). Resistant starch, large bowel fermentation and a broader perspective of prebiotics and probiotics. Benef Microbes 1(4):423-431.

Bird AR, Vuaran M, Brown I, Topping DL (2007). Two high-amylose maize starches with different amounts of resistant starch vary in their effects on fermentation, tissue and digesta mass accretion, and bacterial populations in the large bowel of pigs. Br J Nutr 97(1):134-144.

Brown IL (1994). High amylose starches - new developments in human nutrition. Proc Nutr Soc Austral 18:33-39.

CDC (1992). The Management of Acute Diarrhea in Children: Oral Rehydration, Maintenance, and Nutritional Therapy. U.S. Department of Health and Human Services Public Health Service Centers for Disease Control and Prevention (CDC) Atlanta, Georgia 30333. 41(RR-16);001. Available at: http://www.cdc.gov/mmwr/preview/mmwrhtml/00018677.htm

CDC (2003). *Managing Acute Gastroenteritis Among Children Oral Rehydration, Maintenance, and Nutritional Therapy*. (Morbidity and Mortality Weekly Report (MMWR), vol 52, no RR-16). Atlanta (GA): Centers for Disease Control and Prevention (CDC). Available at: http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5216a1.htm.

Clarke JM, Bird AR, Topping DL, Cobiac L (2007). Excretion of starch and esterified short-chain fatty acids by ileostomy subjects after the ingestion of acylated starches. Am J Clin Nutr 86(4):1146-1151.

Cummings JH, Gibson GR, Macfarlane GT (1989). Quantitative estimates of fermentation in the hind gut of man. Acta Vet Scand Suppl (86):76-82.

de Sales PM, de Souza PM, Simeoni LA, de Oliveira Magalhães P, Silveira D (2012). α-Amylase inhibitors: a review of raw material and isolated compounds from plant source. J Pharm Pharm Sci 15(1):141-183.

De Schrijver R, Vanhoof K, Vande Ginste J (1999). Effect of enzyme resistant starch on large bowel fermentation in rats and pigs. Nutr Res 19(6):927-936.

Elliott EJ, Da Cunha Ferreira RM, Cameron D, Farthing MJ, Walker-Smith JA (1989). Evaluation of three oral rehydration solutions designed for use in developed communities. Aliment Pharmacol Ther 3(3):233-243.

ESPGAN Working Group (1992). Recommendations for composition of oral rehydration solutions for the children of Europe. Report of an ESPGAN Working Group. J Pediatr Gastroenterol Nutr 14(1):113-115.

FAO/WHO (1998). Dietary fibre and resistant starch analysis. In: *Carbohydrates in Human Nutrition*. Report of a Joint FAO/WHO Expert Consultation, Apr. 14-18, 1997, Rome, Italy. (FAO Food and Nutrition Paper no. 66). Rome, Italy: Food and Agriculture Organization of the United Nations (FAO) / Geneva, Switz.: World Health Organization (WHO). Available at: http://www.fao.org/docrep/W8079E/W8079E00.htm.

FAO/WHO (2009). *Principles and Methods for the Risk Assessment of Chemicals in Food: Annex 2. Dose Conversion Table.* (Environmental Health Criteria 240, Annex 2). Geneva, Switz: Food and Agriculture Organization of the United Nations (FAO) / World Health Organization (WHO), International Programme on Chemical Safety (IPCS). Available at: http://whqlibdoc.who.int/ehc/WHO_EHC_240_14_eng_Annex2.pdf.

Farthing M, Salam MA, Lindberg G, Dite P, Khalif I, Salazar-Lindo E *et al.* (2013). Acute diarrhea in adults and children: a global perspective. J Clin Gastroenterol 47(1):12-20.

FASEB (1979). *Evaluation of the Health Aspects of Starch and Modified Starches as Food Ingredients*. (PB80-128804; SCOGS-115; FDA/BF- 80/44). Prepared by Bethesda (MD): Federation of American Societies for Experimental Biology (FASEB) for Washington (DC): Food and Drug Administration, U.S. (FDA), Bureau of Foods.

FCC (2014). Food starch, unmodified. In: *Food Chemicals Codex, 9th edition*. Rockville (MD): United States Pharmacopeial Convention (USP), pp. 499-500.

Ferguson LR, Tasman-Jones C, Englyst H, Harris PJ (2000). Comparative effects of three resistant starch preparations on transit time and short-chain fatty acid production in rats. Nutr Cancer 36(2):230-237.

Filer LJ Jr (1988). Modified food starch—an update. J Am Diet Assoc 88(3):342-344.

Fujita S, Glover DV, Okuno K, Fuwa H (1989). *In vitro* and *in vivo* digestion of high-amylose type starch granules. Starke [Starch] 41(6):221-224.

Grabitske HA, Slavin JL (2009). Gastrointestinal effects of low-digestible carbohydrates. Crit Rev Food Sci Nutr 49(4):327-360.

Gregorio GV, Gonzales MLM, Dans LF, Martinez EG (2009). Polymer-based oral rehydration solution for treating acute watery diarrhoea. In: *Cochrane Database of Systematic Reviews*, Issue 2. Art. No.: CD006519. DOI: 10.1002/14651858.CD006519.pub2.

Guarino A, Albano F, Ashkenazi S, Gendrel D, Hoekstra JH, Shamir R *et al.* (2008). European Society for Paediatric Gastroenterology, Hepatology, and Nutrition/European Society for Paediatric Infectious Diseases evidence-based guidelines for the management of acute gastroenteritis in children in Europe. J Pediatr Gastroenterol Nutr 46(Suppl. 2):S81-S122.

Guiraldes E, Triviño X, Hodgson MI, Quintana JC, Quintana C (1995). Treatment of acute infantile diarrhoea with a commercial rice-based oral rehydration solution. J Diarrhoeal Dis Res 13(4):207-211.

Hall JE (2011). Chapter 65. Physiology of gastrointestinal disorders. In: *Guyton and Hall Textbook of Medical Physiology, 12th edition*. Philadelphia (PA): W.B. Saunders Company, Elsevier.

Hijova E, Chmelarova A (2007). Short chain fatty acids and colonic health. Bratisl Lek Listy 108(8):354-358.

Holtug K, Clausen MR, Hove H, Christiansen J, Mortensen PB (1992). The colon in carbohydrate malabsorption: short-chain fatty acids, pH, and osmotic diarrhoea. Scand J Gastroenterol 27(7):545-552.

ICH (1998). *Ethnic Factors in the Acceptability of Foreign Clinical Data E5(R1) Current Step 4 version dated 5 February 1998 (including the Post Step 4 corrections agreed by the Steering Committee on 11 March 1998)*. (ICH Harmonised Tripartite Guideline). Geneva, Switz.: International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceutical for Human Use (ICH). Available at: http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Efficacy/E5_R1/Step4/E5_R1__Guideline.pdf.

IOM (2005). Dietary, functional, and total fiber. In: *Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein and Amino Acids (Macronutrients)*. (National Academy of Sciences (NAS), Institute of Medicine (IOM), Food and Nutrition Board (FNB), Panel on Micronutrients, Panel on the Definition of Dietary Fiber, Subcommittee on Upper Reference Levels of Nutrients, Subcommittee on Interpretation and Uses of Dietary Reference Intakes, Standing Committee on the Scientific Evaluation of Dietary Reference Intakes). Washington (DC): National Academy Press (NAP), pp. 339-421. Available at: http://www.nap.edu/openbook.php?record_id=10490&page=339.

JECFA (1969). Amylose and amylopectin. In *Toxicological Evaluation of Some Food Colours, Emulsifiers, Stabilizers, Anti-Caking Agents and Certain Other Substances*. 3th Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), May 27-June 4, 1969. (FAO Nutrition Meetings Report Series, no 46A; WHO/FOOD ADD/70.36). Rome, Italy: Food and Agriculture Organization of the United Nations (FAO) / Geneva, Switz.: World Health Organization (WHO). Available at: http://www.inchem.org/documents/jecfa/jecmono/v46aje21.htm.

JECFA (1974). Amylose and amylopectin. In *Toxicological Evaluation of Some Food Additives Including Anticaking Agents, Antimicrobials, Antioxidants, Emulsifiers and Thickening Agents*. 17th Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), June 25-July 4, 1973, Geneva, Switz. (WHO Food Additives Series no 5). Rome, Italy: Food and Agriculture Organization of the United Nations (FAO). Available at: http://www.inchem.org/documents/jecfa/jecmono/v05je65.htm.

JECFA (1982). 3.1.8. Thickening agents: Modified starches. In: *Evaluation of Certain Food Additives and Contaminants*. 26th Report of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), Apr. 19-28, 1982, Rome, Italy. (WHO Technical Report Series, no 683). Geneva, Switz.: World Health Organization (WHO), pp. 29-30, 42, 48. Available at: http://whqlibdoc.who.int/trs/WHO_TRS_683.pdf.

Kaiser P, Borte M, Zimmer KP, Huppertz HI (2012). Complications in hospitalized children with acute gastroenteritis caused by rotavirus: a retrospective analysis. Eur J Pediatr 171(2):337-345.

Kasaoka S, Morita T, Ikai M, Oh-Hashi A, Kiriyama S (1999a). Effect of high-amylose cornstarch on faecal excretion in rats. Nippon Eiyo Shokuryo Gakkaishi [J Jap Soc Nutr Food Sci] 52(5):263-270.

Kasaoka S, Morita T, Hase K, Kiriyama S (1999b). Psyllium regulates the colonic fermentation of high-amylose cornstarch and increases butyrate concentration in the distal colon and feces of rats. Nippon Eiyo Shokuryo Gakkaishi [J Jap Soc Nutr Food Sci] 52(6):365-372.

Kelly DG, Nadeau J (2004). Oral rehydration solution: a “low-tech” oft neglected therapy [Nutrition Issues in Gastroenterology, Series #21]. Pract Gastroenterol 21(10):51-62. Available at: http://www.oley.org/documents/ORS%20Article%20-%20Dr.%20Kelly.pdf.

Kendall CWC, Jenkins DJA, Emam A (2003). Assessment of resistant starch tolerance: a dose response study. Ann Nutr Metab 47(6):636 [abstract S636].

Kopic S, Geibel JP (2010). Toxin mediated diarrhea in the 21 century: the pathophysiology of intestinal ion transport in the course of ETEC, *V. cholera* and rotavirus infection. Toxins (Basel) 2(8):2132-2157.

Le Leu RK, Brown IL, Hu Y, Young GP (2003). Effect of resistant starch on genotoxin-induced apoptosis, colonic epithelium, and lumenal contents in rats. Carcinogenesis 24(8):1347-1352.

Liu H, Ramsden L, Corke H (1997). Physical properties and enzymatic digestibility of acetylated *ae*, *wx*, and normal maize starch. Carbohydr Polym 34(4):283-289.

McClean P, Weaver LT (1993). Ontogeny of human pancreatic exocrine function. Arch Dis Child 68(Spec No. 1):62-65.

Muir JG, Birkett A, Brown I, Jones G, O'Dea K (1995). Food processing and maize variety affects amounts of starch escaping digestion in the small intestine. Am J Clin Nutr 61(1):82-89.

Murphy MM, Douglass JS, Birkett A (2008). Resistant starch intakes in the United States. J Am Diet Assoc 108(1):67-78.

Newberne PN, Conner MW, Estes P (1988). The influence of food additives and related materials on lower bowel structure and function. Toxicol Pathol 16(2):184-197.

Nugent AP (2005). Health properties of resistant starch. Nutr Bull BNF 30:27-54.

Phillips J, Muir JG, Birkett A, Lu ZX, Jones GP, O'Dea K *et al.* (1995). Effect of resistant starch on fecal bulk and fermentation-dependent events in humans. Am J Clin Nutr 62(1):121-130.

Raghupathy P, Ramakrishna BS, Oommen SP, Ahmed MS, Priyaa G, Dziura J *et al.* (2006). Amylase-resistant starch as adjunct to oral rehydration therapy in children with diarrhea. J Pediatr Gastroenterol Nutr 42(4):362-368.

Ramakrishna BS, Subramanian V, Mohan V, Sebastian BK, Young GP, Farthing MJ *et al.* (2008). A randomized controlled trial of glucose versus amylase resistant starch hypo-osmolar oral rehydration solution for adult acute dehydrating diarrhea. PLoS ONE 3(2):e1587. doi 1510.1371/journal.pone.0001587.

Ramakrishna [BS] (2012) [unpublished]. *Review of CSIRO Research with Acetylated Starplus™ [Confidential].* (Starplus Experimental Review). Prepared by Vellore, India: Christian Medical College and Hospital for Canberra, Australia: The Commonwealth Scientific and Industrial Research Organisation (CSIRO), Indo-Australian Biotechnology Grant Proposal.

Richardson PH, Jeffcoat R, Shi Y-C (2000). High amylose starches from biosynthesis to their use as food ingredients. MRS Bull (Dec.):20-24.

Saito K, Ito T, Kuribayashi T, Mochida K, Nakakuki T, Shibata M *et al.* (2001). Effect of raw and heat-moisture treated high-amylose corn starch on fermentation by the rat cecal bacteria. Starke [Starch] 53(9):424-430.

Sandstedt RM, Strahan D, Ueda S, Abbot RC (1962). The digestibility of high-amylose corn starches compared to that of other starches. The apparent effect of the *ae* gene on susceptibility to amylase action. Cereal Chem 39:123-131.

Scheppach W (1994). Effects of short chain fatty acids on gut morphology and function. Gut 35(1, Suppl.):S35-S38.

Topping DL, Clifton PM (2001). Short-chain fatty acids and human colonic function: Roles of resistant starch and nonstarch polysaccharides. Physiol Rev 81(3):1031-1064.

U.S. FDA (1993). Appendix I. Table 14. Conversion table for test chemical treatment doses used in PAFA. In: *Priority Based Assessment of Food Additives (PAFA) Database*. Washington (DC): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN), p. 58. Available at: http://www.fda.gov/ohrms/DOCKETS/DOCKETS/95s0316/95s-0316-Rpt0272-36-Appendix-D-Reference-F-FDA-vol205.pdf.

U.S. FDA (1996). Regulation of medical foods: Advance notice of proposed rulemaking (21 CFR Ch. I. [Docket No. 96N–0364] RIN 0905–AD91). Fed Regist (US) 61(231):60661-60671.

U.S. FDA (2006). Chapter 21 – Food composition, standards, labeling and economics. Medical Foods Program – Import and domestic (FY 06/07/08). In: *Compliance Program Guidance Manual*. (Program 7321.002). College Park (MD): U.S. Food and Drug Administration (U.S. FDA). Available at: http://www.fda.gov/downloads/Food/ComplianceEnforcement/UCM073339.pdf.

U.S. FDA (2011). *The Straight Poop on Kids and Diarrhea*. (FDA Consumer Heal t h Information, Oct. 2011). College Park (MD): U.S. Food and Drug Administration (U.S. FDA). Available at: http://www.fda.gov/forconsumers/consumerupdates/ucm277359.htm [Page Last Updated: 11/15/2013].

UNICEF/WHO (2009). *Diarrhoea: Why Children are Still Dying and What Can be Done*. New York (NY): United Nations Children's Fund (UNICEF) / Geneva, Switz.: World Health Organization (WHO). Available at: http://www.who.int/maternal_child_adolescent/documents/9789241598415/en/.

van Munster IP, Tangerman A, Nagengast FM (1994a). Effect of resistant starch on colonic fermentation, bile acid metabolism, and mucosal prolife. Dig Dis Sci 39(4):834-842.

van Munster IP, de Boer HM, Jansen MC, de Haan AF, Katan MB, van Amelsvoort JM *et al.* (1994b). Effect of resistant starch on breath-hydrogen and methane excretion in healthy volunteers. Am J Clin Nutr 59(3):626-630.

Vesikari T, Isolauri E, Baer M (1987). A comparative trial of rapid oral and intravenous rehydration in acute diarrhoea. Acta Paediatr Scand 76(2):300-305.

Wang Y-J, White P, Pollak L, Jane J (1993). Characterization of starch structures of 17 maize endosperm mutant genotypes with Oh43 inbred line background. Cereal Chem 70(2):171-179.

WGO (2012). *Acute Diarrhea in Adults and Children: a Global Perspective*. (Official Guidelines). Munich, Germany: World Gastroenterology Organisation (WGO). Available at: http://www.worldgastroenterology.org/assets/export/userfiles/Acute%20Diarrhea_long_FINAL_120604.pdf.

WHO (2004). *First Steps for Managing an Outbreak of Acute Diarrhoea: Leaflet*. (WHO/CDS/CSR/NCS/2003.7 Rev.1). Geneva, Switz.: World Health Organization (WHO), Global Task Force on Cholera Control. Available at: http://www.who.int/cholera/publications/firststeps/en/.

WHO (2005). *The Treatment of Diarrhoea: a Manual for Physicians and Other Senior Health Workers*. (4th rev: WHO/CDD/SER/80.2). Geneva, Switz.: World Health Organization (WHO). Available at: http://www.who.int/maternal_child_adolescent/documents/9241593180/en/.

SUBMISSION END