

ORIGINAL SUBMISSION

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OFFICE OF HEALTH AND HUMAN SERVICES
DEPARTMENT OF FOOD AND DRUG ADMINISTRATION
FOOD ADDITIVE SAFETY**GENERALLY RECOGNIZED AS SAFE
(GRAS) NOTICE**Form Approved: OMB No. 0910-0342; Expiration Date: 02/29/2016
(See last page for OMB Statement)**FDA USE ONLY**

GRN NUMBER

000616

DATE OF RECEIPT

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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 (Check one)

amendment or supplement submitted in
response to a communication from FDA?☐ Yes

If yes, enter the date of

☐ No

communication (yyyy/mm/dd): _____

PART II – INFORMATION ABOUT THE NOTIFIER**1a. Notifier**

Name of Contact Person

Henry J. Binder, MD

Position

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

New Haven

State or Province

Connecticut ▼

Zip Code/Postal Code

06519

Country

United States of America ▼

Telephone Number

203-785-4796

Fax Number

E-Mail Address

**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

ACETYLATED 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	1.Acetylated HAMS (amylose (MS), a high amylose maize starch (HAMS, Hi-maize™; 85% amylose)				
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 starch

control 3: amylase-resistant starch

3. Synonyms

Provide as available or relevant:

1	Acetylated HAMS; Crispfilm, Eurylon, Amylacetate, Batter Crisp; C*Batter-Crisp; Gelose
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

ACTIVATED CHARCOAL

(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. ☐ _____ 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.

(name of notifier)

(address of notifier or other location)

_____ agrees to send these data and information to FDA if FDA asks to do so.

(name of notifier)

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

(b) (6)

Printed Name and Title

S M Girvin, Deputy Provost

Date (mm/dd/yyyy)

12/7/15

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)
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Documentation Supporting the Determination that High-Amylose Maize Starch Acetate (HAMSA2.5) is Generally Recognized as Safe (GRAS) for Use as an Ingredient in Oral Rehydration Solutions that are Medical Foods

Yale University
New Haven
CT 06520 USA

Prepared by:

Intertek Scientific & Regulatory Consultancy
2233 Argentia Road, Suite 308
Mississauga, Ontario, Canada
L5N 2X7
www.intertek.com

March 25, 2015

Documentation Supporting the Determination that High-Amylose Maize Starch Acetate (HAMSA2.5) is Generally Recognized as Safe (GRAS) for Use as an Ingredient in Oral Rehydration Solutions

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Documentation Supporting the Determination that High-Amylose Maize Starch Acetate (HAMSA2.5) is Generally Recognized as Safe (GRAS) for Use as an Ingredient in Oral Rehydration Solutions

1.0 INTRODUCTION

Yale University (Yale) intends to market a new oral rehydration solution (ORS) that contains resistant starch, specifically acetylated (2.5%) high-amylose maize starch (HAMSA2.5), as a replacement for glucose. Hypo-osmolar glucose-based solutions are currently endorsed by 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 starch acetate (INS No. 1420) is a widely accepted food ingredient/additive 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 HAMSA2.5 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 HAMSA2.5, (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 HAMSA2.5 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 HAMSA2.5 as a replacement for glucose in ORS. Information pertaining to the composition and manufacturing process for HAMSA2.5, as well as batch analyses and intended uses are presented.

The data and information summarized in this dossier demonstrate that HAMSA2.5, 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 HAMSA2.5

2.1 Common or Usual Name

Acetylated (2.5%) high-amylose maize starch; acetylated (2.5%) high-amylose corn starch; starch acetate, acetyl starch

2.2 Chemical Name

Not applicable.

2.3 Chemical Abstract Service (CAS) Number

The CAS number for starch acetate is 9045-28-7.

2.4 Physical and Chemical Characteristics

2.4.1 Physical Properties

In general, modified food starches are insoluble in alcohol, ether and chloroform (JECFA, 2013; FCC, 2014). They are also insoluble in cold water, unless they are treated with to be pregelatinized or made cold-water swelling (JECFA, 2013; 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) (JECFA, 2013; 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). Acetylated starches have technological functions as film-forming, binding, adhesion, thickening, stabilizing, and texturing agents in a variety of foods (Luo and Shi, 2012).

2.4.2 Chemical Properties

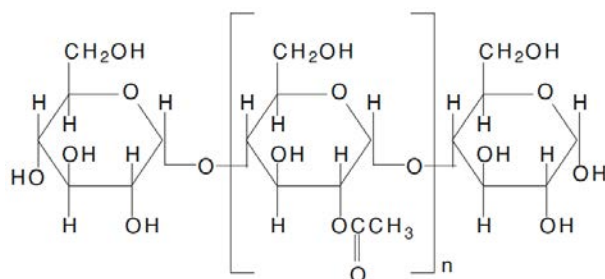
Starches are polysaccharides, and they are the major dietary source of carbohydrates (Sajilata *et al.*, 2006). There are 2 main structural components of starch; amylose is a linear polymer of glucose residues bound *via* α -D-(1,4)-glycosidic linkages, while amylopectin is a highly branched molecule 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). Native food starches may be chemically modified to improve the physicochemical and functional properties of the starch (BeMiller, 2009). Currently, the acetylated starches used in the food industry is produced *via* treatment with acetic anhydride or

vinyl acetate, and the proportion of acetyl group introduced into the finished product should not exceed 2.5%, which corresponds to a maximum degree of substitution¹ of 0.1 (JECFA, 1982a, 2013; FCC, 2014). The introduction of acetyl groups may disrupt interactions among the amylopectin and amylose molecules, thereby altering the functional characteristics of the native starch (e.g., conferring higher peak viscosity, paste clarity, and increasing freeze-thawed stability) (Liu *et al.*, 1997; Wickramasinghe *et al.*, 2009).

HAMSA2.5 is produced by the acetylation of high-amylose maize starch (HAMS). 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 50 to 80% amylose (Sajilata *et al.*, 2006; BeMiller, 1973).

Structural Formula:

A) Example of an acetylated glucose polymer



3.0 METHOD OF MANUFACTURE

3.1 Manufacturing Process

A schematic overview of the manufacturing process of HAMSA2.5 is presented in Figure 3.1-1. HAMSA2.5 is manufactured using processes similar to those commonly employed in the industry to produce modified food starches (U.S. EPA, 1994). HAMSA2.5 is obtained by the esterification of high-amylose maize starch (Hylon-VII®) with acetate, *via* treatment with acetic

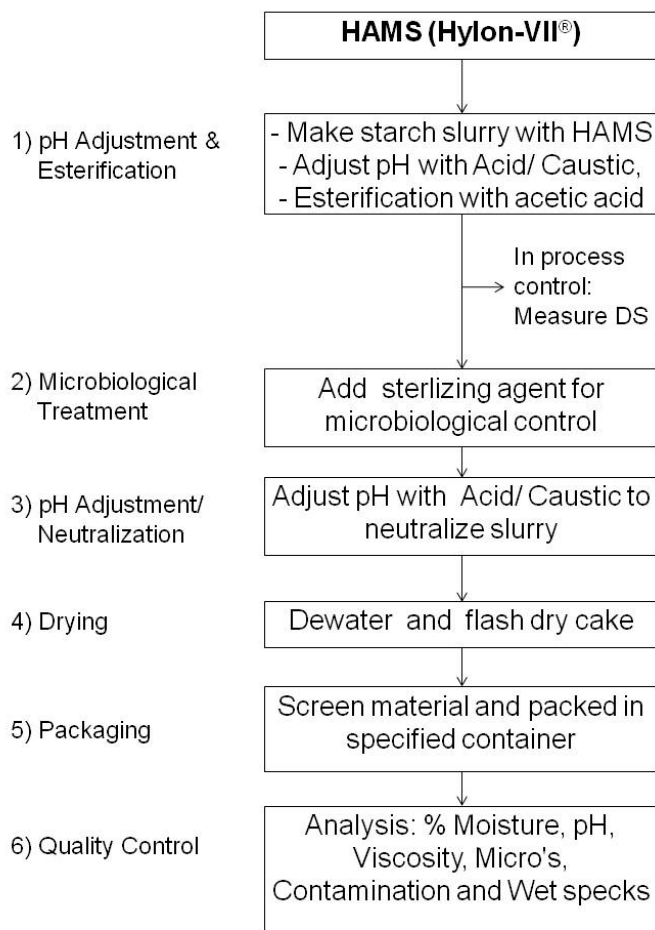
¹ The acetyl content (A) of acetylated starch can be determined titrimetrically according to the methods of Wurzburg (1964). The degree of substitution (DS) can be calculated from the measured acetyl content using the following formula:

$$DS = \frac{162 \times A}{43 \times 100 - (43 - 1) \times A}$$

The DS can also be measured using direct methods such as NMR spectroscopy or FTIR (Alissandratos *et al.*, 2010).

acid. The HAMSA2.5 ingredient intended for use in ORS is manufactured in accordance with cGMP, and it has been marketed as a food ingredient in the U.S. and globally for many years.

Figure 3.1-1 Schematic Overview of the Manufacturing Process for Acetylated (2.5%) High-Amylose Maize Starch (HAMSA2.5)



3.2 Product Specifications

The HAMSA2.5 ingredient intended for use in ORS meets the specifications for food-grade acetylated starch, as defined in the Food Chemicals Codex (FCC) and the Joint FAO/WHO Expert Committee on Food Additives (JECFA) monographs for modified starches (JECFA, 2013; FCC, 2014). These specifications are presented in Table 3.2-1.

Table 3.2-1 Food-Grade Specifications for Acetylated (2.5%) High-Amylose Maize Starch		
Parameter	Specification for Acetylated Starch (INS No. 1420)	
	FCC (2014)	JECFA (2013)
Process method	Treatment with acetic anhydride or vinyl acetate	Treatment with acetic anhydride or vinyl acetate
Proportion (%) of acetyl groups in the final product	NMT 2.5	NMT 2.5
Identity		
Iodine stain	Positive (dark blue to red color)	Positive (dark blue to red color)
Copper reduction	Copious red precipitate forms	Copious red precipitate forms
Microscopy	Typical polarization cross	Typical polarization cross
Crude fat (%)	NMT 0.15	--
Protein (%)	NMT 1 for high amylase-starch	--
Loss on drying (%)	NMT 15.0	--
pH	3.0 to 9.0	--
Lead (mg/kg)	NMT 1	NMT 2
Sulfur dioxide (mg/kg)	NMT 50	NMT 50

NMT = not more than

3.3 Batch Analysis

Analysis of 4 non-consecutive lots of HAMSA2.5 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 HAMSA2.5 is presented in Table 3.3-1 (see Appendix A for Certificates of Analysis).

Table 3.3-1 Batch Analysis Data for 4 Non-Consecutive Lots of Acetylated (2.5%) High-Amylose Maize Starch (HAMSA2.5)				
Parameter	Manufacturing Lot			
	ACK5018	ECK5245	HCK5395	CCK5094
pH	5.62	6.32	5.13	5.65
Moisture (%)	11.16	11.68	13.13	11.71
% through 100 mesh	99.98	100.00	99.86	99.92
% through 200 mesh	98.63	98.42	98.18	98.19
% on 35 mesh	0.00	0.00	0.00	0.00
Heavy Metals				
Lead (mg/kg)	0.00685	<0.005	<0.005	Not conducted
Microbial Contamination				
Total plate count	<10	565	500	410
Yeast	<10	0	10	<10
Mold	<10	40	10	<0
<i>Escherichia coli</i>	Negative	Negative	Negative	Negative
Salmonella	Negative	Negative	Negative	Negative

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 Bjork, 1992; Champ, 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 (RS₂), 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 (RS₄) (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 (RS₁); indigestible starch that is formed by retrogradation upon gelatinization (RS₃); and amylose-lipid complexed starch (RS₅) (Brown, 1994; Topping *et al.*, 2003; Sajilata *et al.*, 2006; Slizewska *et al.*, 2012). The resistant starch content of HAMSA2.5 has been estimated at 74% (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 HAMSA2.5, 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 HAMSA2.5 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 HAMSA2.5, there were no notable changes in the chemical or microbiological parameters of a manufacturing batch of HAMS (Hylon-VII®), the starting material of HAMSA2.5, 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
<i>Escherichia coli</i>	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

4.1 Current Regulatory Status

4.1.1 Acetylated High-Amylose Maize Starch

Acetylated forms of native food starches (including those extracted from hybrid crops such as high-amylose maize) are widely accepted food additives that are used in the food supply globally. Starch acetate (INS No. 1420), produced by esterification of food starch with acetic anhydride or vinyl acetate, with the acetyl groups not exceeding more than 2.5% in the final product (*i.e.*, HAMSA2.5), is included in Table 3 of the Codex General Standard for Food Additives (CODEX, 2013). This means that starch acetate may be added to foods in general (with the exception of the food categories that are listed in the Annex to Table 3), at *quantum satis* levels in accordance with the principles of Good Manufacturing Practice (GMP) (CODEX, 2013). JECFA has concluded the Acceptable Daily Intake (ADI) level for starch acetate to be “not specified”, a designation that is assigned 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, 1982a,b).

In the U.S., food starches that have been esterified by treatment with acetic anhydride or vinyl acetate, such that the acetyl groups in the modified food starch does not exceed 2.5%, are food additives that are permitted for the direct addition to food for human consumption (21 CFR §172.892 – U.S. FDA, 2013b). Aside from adherence to GMP (*i.e.*, the amount added does not exceed those reasonably required to accomplish the intended physical or technical effect), there are no other limitations on the use of these acetylated food starches in foods (21 CFR §172.892 – U.S. FDA, 2013b). Starch acetate is also permitted for use in foods in general without any restrictions on their use, aside from adherence to GMP, in many other jurisdictions (*e.g.*, Canada, Europe, and Australia/New Zealand) (EU, 2011; Health Canada, 2012; FSANZ, 2013).

4.1.2 Oral Rehydration Solution (ORS)

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 several authoritative bodies have indicated that rice-based ORS may be recommended for these cases (WHO, 2005; 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 A2.5 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 nucleotides 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 of diarrhea can vary from 5 mL/kg (minimal dehydration) to over 200 mL/kg (WHO, 2005).

4.2.2 Target Population

ORS containing HAMSA2.5 as a carbohydrate source is intended for use in children (>2 years of age) and adults with acute diarrhea, with or without signs of mild to moderate dehydration², 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 Solution

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

² 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).

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 Prevention³ (CDC) (CDC, 2003; WHO, 2005; WGO, 2012). These recommendations are summarized in Table 4.2.3-1 below.

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 only 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).

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, 2004, 2005	WGO, 2012	CDC, 2003
Children (age not specified)	75 mL/kg bw over the first 4 h ^b , providing up to 20 mL/kg bw/h	50 to 100 mL/kg bw over the first 3 to 4 h	50 to 100 mL/kg bw over the first 3 to 4 h
Adults	75 mL/kg bw during the first 4 h, providing up to 750 mL/h	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
Infants and toddlers (<2 yrs or <10 kg)	50 to 100 mL, up to 500 mL/day	50 to 100 mL	60 to 120 mL
Children	100 to 200 mL, up to 1 L/day (2 to 10 yrs)	Amount not specified	120 to 240 mL (>10 kg)
Adults	As much as needed, up to 2L/day	Amount not specified	Recommendations not provided

bw = body weight; ORS = oral rehydration 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

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).

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); 800 to 1,200 mL (2 to 4 years); 1,200 to 2,200 mL (5 to 14 years); and 2,200 to 4,000 mL (≥15 years).

4.2.4 Use Levels of HAMSA2.5 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 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 ESGPHAN also recommends a “hypotonic osmolarity solution” that contains 60 mmol/L of sodium (Na⁺) (Guarino *et al.*, 2008). Yale intends to add HAMSA2.5 at levels of 50 g/L as a nutrient ingredient 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
ESGPHAN Formula	16 g/L carbohydrate or 74 to 111 mmol/L (glucose)	60	20	60	10 (30 mM base)	200 to 250
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 HAMSA2.5	50 g/L (HAMSA2.5)	-	-	-	-	-

HAMSA2.5 = acetylated (2.5%) high-amylose maize starch; ORS = oral rehydration solution

^a Data taken from ESPGAN, 1992; WHO, 2002; CDC, 2003; UNICEF, 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 (C₆H₅O₇) 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 HAMSA2.5 FROM ITS USE IN ORAL REHYDRATION SOLUTION

5.1 Background Dietary Intakes

According to the review conducted by the Select Committee on GRAS Substances (SCOGS) on the health aspects of starch and modified starches as food ingredients, the intake of modified starches by the total U.S. population (age 2 to 65 years) was estimated to be 11.8 g/day, based on the typical use levels of modified starches in foods, and the intake of these foods collected from dietary surveys. However, the SCOGS did note that this was likely to be an overestimation due to the conservative assumptions made (e.g., modified starches will be added to all food categories at the maximum levels, and that all of these foods are consumed on a daily basis), and further indicated that the intake of modified starches, based on poundage data of the amount that is sold annually, was approximately 0.7 to 1 g/person/day (FASEB, 1979).

Additionally, 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 classified as “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 Intake⁴ 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),

⁴ 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).

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 in the U.S., Europe, and Australia/New Zealand 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 5.1-1. 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).

Table 5.1-1 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 <i>et al.</i> , 2008
Europe	In 10 different countries ^b Mean: 4.1; Range: 3 to 6	Dyssler and Hoffem, 1995
Australia/New Zealand	Minimum (mean $\pm$ SEM) ^c : 3.4 $\pm$ 0.03 Maximum (Mean $\pm$ SEM) ^c : 9.4 $\pm$ 0.07	Roberts <i>et al.</i> , 2004
India	Mean ^d : 10	Platel and Shurpalekar, 1994
China	Mean $\pm$ SEM ^e : 18 $\pm$ 1.1	Muir <i>et al.</i> , 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).

^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 HAMSA2.5 from Uses in Oral Rehydration Solution

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 intake of HAMSA2.5 that would be expected, based on the recommendations of the authoritative bodies, are summarized in Table 5.2-1.

Table 5.2-1 Estimated Intake of HAMSA2.5 Based on Recommended Uses of Oral Rehydration Solution by Authoritative Bodies for Managing Dehydration During Acute Diarrhea						
Age	Weight (kg)	Rehydration Phase (First 4 Hours)		Maintenance Phase		Intake of HAMSA2.5 (g/day) ^d
		Amount of ORS (mL) ^a	Intake of HAMSA2.5 (g) ^b	Recommended Amount of ORS (mL/day) ^c	Intake of HAMSA2.5 (g/day) ^c	
<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 1090	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 1,000	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

HAMSA2.5 = acetylated (2.5%) high-amylose maize starch; 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 (CDC, 2003; WHO, 2005; 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); 1,200 to 2,200 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/hour (i.e., 3,000 mL) (WHO, 2005).

^b HAMSA2.5 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.

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 HAMSA2.5 IN ORAL REHYDRATION SOLUTIONS

6.1 Introduction

HAMSA2.5 and its source material, HAMS, have a long history of safe use in the food supply. High-amylose maize starch is a well accepted food ingredient that has been consumed since the 1950s (BeMiller, 2009), while HAMSA2.5 is a food additive approved for use without any restrictions in many countries (see Section 4.1.1 above). Authoritative bodies such as JECFA and the SCOGS have evaluated the safety of starch acetate (INS No. 1420), and it has been concluded that the current uses of this food additive would not pose any concerns to human health (FASEB, 1979; JECFA, 1982a). Although the food uses of HAMSA2.5 are well-established, this ingredient has not been used previously as the sole carbohydrate source in ORS. Standard glucose-based ORS, as well as cereal-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, the key component of the safety

assessment for the use of HAMSA2.5 as the carbohydrate source in ORS would involve demonstration that such formulations are at least as effective as standard glucose-based ORS formulations in addressing the dehydration associated with diarrhea, and does not further exacerbate the existing diarrhea. The totality of evidence to support the safety of HAMSA2.5 as an ingredient in ORS intended for use by 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, is presented in Section 6.2 to 6.6 below. This includes a discussion of the metabolic fate of HAMSA2.5, toxicological evaluation for acetylated starches, and data from both animal and clinical studies demonstrating that ORS containing HAMSA2.5, or its source material HAMS, would not pose any safety concerns, particularly with respect to adverse gastrointestinal effects.

6.2 Metabolic Fate

6.2.1 Resistant Starches (HAMS)

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 (McClellan 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; Gadjia *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 Acetylated Starches

6.2.2.1 *In vitro* Studies

A) Digestibility by Human Gastrointestinal Enzymes

Acetylation is one processing method by which native food starches can be modified to increase their resistance to digestibility by gastrointestinal enzymes, thereby increasing the content of resistant starch (RS₄) (Brown, 1994; Topping *et al.*, 2003; Sajilata *et al.*, 2006; Slizewska *et al.*, 2012). It has been proposed that the reduced degree of hydrolysis by the introduction of acetyl groups was attributed to the steric hindrance imposed by the bulky substituent (Hoover and Sosulski, 1985). Accordingly, a number of *in vitro* studies have indicated that the digestibility of acetylated starch is reduced compared to their native, readily digested forms. For example, in earlier studies that were evaluated by the SCOGS and JECFA, the digestibility of acetylated cornstarch, containing 1.5 and 2.41% acetyl groups, was approximately 84 and 69% of the digestibility of unmodified cornstarch, respectively, following incubation with fungal amyloglucosidase (Turner, 1961; Kruger, 1970). It was also reported that the digestibility of starch acetate (containing 1.98% acetyl groups) by pancreatin and porcine mucosal enzymes was only 90% of that of the unmodified starch (Leegwater, 1971). JECFA noted in 1 study, the digestibility of starch acetate containing 2.5% acetyl groups was reported at approximately 93.7% of the native starch (Turner, 1961).

B) Hydrolysis of Acetyl Groups

Studies conducted *in vitro* have provided evidence that acetyl groups are released from acetylated HAMS (HAMSA) by the action of bacterial lipases and/or esterases (Morita *et al.*, 2005; Ramakrishna, 2012). The hydrolysis of acetate from HAMSA appears to be slow, with only approximately 10% of the releasable acetate of HAMSA (DS of 0.18, or approximately 4.8% acetyl groups) being hydrolyzed following 4-hour incubation with bacterial enzymes extracted from rat cecal contents (Morita *et al.*, 2005).

6.2.2.2 *Animal Studies*

In early animal studies that were reviewed by the SCOGS and JECFA, acetylated starch appeared to be digested in a similar manner as native starch, though only limited information on the methodologies (*e.g.*, source of the starch) and endpoints measured were provided in their evaluation reports. The SCOGS has noted that no differences in digestibility were observed in rats fed diets containing acetylated cornstarch (1.8% acetyl groups) compared to administration of a pre-gelatinized cornstarch (Kruger, 1970). Moreover, JECFA indicated that there were no significant differences in the caloric value of diets containing either acetylated starch (1.8% acetyl groups) or native starch that were administered to rats for 4 weeks, suggesting a similar amount of starch was digested and utilized for energy (Oser, 1961).

More recently, Annison *et al.* (2003) demonstrated that acetylation of a readily digested native starch (*i.e.*, low-amylose native starch) does result in reduced digestibility, and increased provision of fermentable substrate to the colon. Adult male Hooded Wistar rats were administered standard diets containing native low-amylose maize starch (LAMS), or low-amylose maize starch that has been acylated with acetate (DS 0.20; LAMSA), propionate (DS 0.16) or butyrate (DS 0.19) (Annison *et al.*, 2003). The diets contained native maize starch at 401.5 g/kg diet, plus 150 g/kg diet [15 g/kg body weight/day (U.S. FDA, 1993)] of 1 of the acylated starches or the control native maize starch for 14 days. Administration of the LAMSA significantly increased the amount of starch in the cecum and distal colon (by approximately 10- and 6-fold, respectively) when compared to animals administered the same amount of LAMS, which does not contain RS and is expected to fully digested. Furthermore, administration of LAMSA significantly increased all short-chain fatty acids (SCFA) (acetate, propionate, and butyrate) in the cecum and colon compared to administration of LAMS, likely as a result of the increased provision of fermentative substrate to the colon. Acetate levels were increased to the greatest extent when compared to the other SCFA. Accordingly, the pH in the cecum and colon were also significantly decreased.

Studies have also been conducted to specifically investigate the impact of acetylation towards the digestibility and fermentative capacity of HAMS, a naturally occurring RS. Morita *et al.* (2005) conducted an experiment where colectomized male Sprague-Dawley rats were

administered diets containing 655 g/kg diet of standard maize starch, HAMS, or HAMSA (DS of 0.18, or approximately 4.8% acetyl groups) for 3 days, corresponding to doses of approximately 65.5 g/kg body weight/day (U.S. FDA, 1993). The ileal excreta was collected during this time. The ileal digestibility of the starches, based on the amount of undigested starch collected from the terminal ileum, was significantly lower for HAMSA (at 47.5%) compared to HAMS (at 58%), though both were much less than the digestibility of standard maize starch (at 99.8%), for which digestion was virtually complete.

Additional experiments conducted by Morita *et al.* (2005) in intact animals indicate that acetate is hydrolyzed from HAMSA in the large intestines, further contributing to the pool of SCFA that are generated from the fermentation of the liberated starch. In rats fed a commercially available diet containing 4% crude fiber, administration of HAMSA at 300 g/kg diet [*i.e.*, 30 g/kg body weight/day (U.S. FDA, 1993)] for 14 days resulted in significantly increased cecal content of acetate, total SCFA, and cecal weight, while cecal pH was significantly decreased, in comparison to administration of the same amount of HAMS. In another similar experiment, Sprague-Dawley rats were fed a fiber-free purified diet containing 655 g/kg diet of maize starch (control), HAMSA (DS of 0.18, or approximately 4.8% acetyl groups) at 100, 200, or 300 g/kg diet [*i.e.*, 10, 20, or 30 g/kg body weight/day (U.S. FDA, 1993)] or HAMS at 200 g/kg diet [*i.e.*, 20 g/kg body weight/day (U.S. FDA, 1993)] as substitution for the maize starch for 14 days. Administration of HAMSA resulted in a dose-dependent increase in the cecal content of acetate, the total amount of SCFA, and cecal weight, as well as dose-dependent decrease in pH. Furthermore, the amount of acetate and total amount of SCFA were significantly higher (by approximately 2-fold), while the pH of the cecal content was significantly lower, in animals fed diets containing an equivalent amount of HAMSA as HAMS (*i.e.*, 20 g/kg body weight/day). Notably, there was also a significant increase in the fecal excretion of undigested starch (737 vs. 192 mg/day) and fecal dry matter (1.41 vs. 0.75 g/day) in animals receiving HAMSA as those receiving an equivalent amount of HAMS. The authors proposed that the increased fecal starch excretion may have represented an inability of the microflora to completely hydrolyze and/or ferment the influx of RS, though it is unclear whether this effect was related to feeding of a highly purified diet, which may have limited the diversity of the microflora population, rather than representing a specific attribute of the HAMSA or HAMS.

In another study, male Hooded Wistar rats were fed diets containing standard maize starch or HAMS at 550 g/kg diet, or diets containing 150 g/kg [approximately 15 g/kg body weight/day (U.S. FDA, 1993)] of an industrially or laboratory synthesized HAMSA (DS 0.2) plus 400 g/kg diet of HAMS for 7 days (Bird *et al.*, 2006). The “laboratory synthesized” HAMSA was produced using the same methods as those used by the Annison *et al.* (2003) study (*i.e.*, acetylation by acetic anhydride with methylimidazole as a catalyst), whereas the “industrially synthesized” HAMSA were same as the methods used in the Morita *et al.* (2005) study (*i.e.*, acetylation by acetic anhydride in the presence of sodium hydroxide and hydrogen peroxide). There were no

significant differences in the wet weight of the cecal contents among rats receiving the different dietary interventions. The amount of acetate and total SCFA in the cecal digesta was significantly higher in laboratory synthesized HAMSA (480 μmol and 551 μmol , respectively), but not the industrially synthesized HAMSA (343 μmol and 483 μmol , respectively), when compared to administration of the HAMS diet only (266 μmol and 339 μmol , respectively). Nevertheless, administration of diets containing HAMSA (either laboratory or industrially synthesized) or HAMS significantly increased the amount of individual and total SCFA in the cecal digesta compared to administration of a standard maize starch alone (41 μmol and 63 μmol , respectively).

The release of acetate from HAMSA is unlikely to require adaptation of the colonic microflora, as administration of acetylated maize starch (low amylose content) as a single dose *via* gavage was reported to significantly increase the individual and total SCFA levels of the cecal digesta starting from 2-hours post-gavage and onwards (Ramakrishna, 2012).

6.2.2.3 Human Studies

Clarke *et al.* (2007) conducted a study to investigate the digestibility of chemically modified starches, including HAMSA, when compared to HAMS in ileostomy subjects. In this cross-over study, 7 ileostomy subjects (6 ♀; 1 ♂; $\bar{x}$ = 56 y) participated in 2 different test periods each consisting of 3 treatment days. On each of these 3 days, the subjects consumed a baked, milk-based, chocolate custard containing 20 g of one of the following starches: low-amylose maize starch (LAMS), HAMS, HAMSA, HAMS propionate (HAMSP), or HAMS butyrate (HAMSB). As there were only 5 different interventions, the LAMS were consumed twice by the subjects (once in each test session), while the other test-articles were consumed only once. For 24 hours prior to the test session and throughout the 3-day testing periods, the subjects were provided a standard Western diet containing low levels of starch. The degrees of substitution of the chemically modified starches were 0.23, 0.24, and 0.25 for HAMSA, HAMSP, and HAMS, respectively. Analysis of the test custards for starch content demonstrated equivalent levels of starch in a single 130 g serving of each test custard. The custards containing HAMSA, HAMSP, and HAMS contained significantly larger amount of the corresponding esterified SCFA (*i.e.*, 25.1, 24.9, and 28.2 mmol/130 g serving of acetic, propionic and butyric acid respectively for HAMSA, HAMSP, and HAMS). In contrast, the LAMS and HAMS test custards contained only trace quantities of SCFA.

All resistant starches were observed to be partially digested in the small intestine. Starch digestibility 72.9% for HAMS, 71.6% for HAMSA, 72.4% for HAMSP and 67.9% for HAMS, which were significantly less than the digestibility of LAMS (94.6%) ($p < 0.001$). No statistically significant differences in digesta wet weight, digesta dry weight or starch output were observed in subjects consuming HAMSA compared to HAMS, providing further demonstration that similar amounts of undigested starch were present at the end of the small intestinal tract. Furthermore,

no significant differences in digesta pH (all values ≥ 7.4) or free SCFA levels in stoma digesta were observed, indicating that minimal fermentation was occurring in the small intestine. The authors noted that 73.9, 76.2, and 73.1% of the esterified SCFA in the custards containing HAMSA, HAMSP, and HAMSB, respectively, survived passage through the small intestines. This suggests that the majority of the acylated starches will not be hydrolyzed to release its SCFA group within the small intestines, but rather, they could potentially deliver additional SCFA to the large intestines. For example, in this study, ingestion of custard containing HAMSA would have delivered an additional 18.5 mmol of acetate to the large intestines (*i.e.*, 0.739×25.1 mmol), compared to ingestion of the same amount of custard containing HAMS.

In a randomized controlled cross-over study, 15 healthy volunteers were administered warm flavored drinks containing HAMS or HAMSA (6% acetylation) at levels of 20 or 40 g/day for 2 weeks (duration of washout period not provided) (Ramakrishna, 2012). Subjects consumed a diet low in RS for 2 weeks before and throughout the course of the study. Consistent with the results of Clarke *et al.* (2007), HAMSA and HAMS delivered equivalent quantities of resistant starch to the large intestine (with total digestibility reported in the range of 93.1 to 99.2%), and there were no significant differences in measurements of fecal output, moisture, pH, and ammonia concentrations in subjects administered HAMSA compared to HAMS. Among subjects consuming 40 g HAMSA/day, the amount of starch excreted in the feces was significantly higher (at 1.15 ± 0.2 g/100 g) compared to the amount of starch excreted following administration of HAMS (0.59 ± 0.2 g/100 g for 20 g/day dose, or 0.62 ± 0.20 g/100 g for 40 g/day dose). As expected, a statistically significant increase in fecal free acetate concentration was observed in volunteers consuming 40 g HAMSA/day (62.1 ± 4.3 $\mu\text{mol/g}$) compared to administration of the control diet during baseline (52.8 ± 8.4 $\mu\text{mol/g}$).

In one cross-over study, 11 healthy male subjects (mean age = 24 years) were administered a pudding containing 50 g of one of the following heated/cooked starches: 1 to 2% acetylated potato starch, native unmodified potato starch, or native potato starch mixed with 2% cyclodextrin (Raben *et al.*, 1997). All of the potato starches contained 26% amylose content, and there was a 3- to 5-week washout in between administration of each of the test meals. For 2 days prior to consuming the test pudding, the subjects were given a carbohydrate-rich, weight-maintenance diet prepared to meet each subject's individual energy requirements. Blood samples were collected at baseline, prior to administration of the test pudding in the morning, followed by post-prandial measurements for 6 hours. Administration of the test diet containing acetylated potato starch did not affect post-prandial glucose or insulin levels compared to the control starch, which suggests acetylation did not greatly impair the digestibility of the starch.

6.2.2.4 Summary

Chemical modification of native food starches introduces structural changes that alter their physicochemical properties (Xu *et al.*, 2004; Ayucitra, 2012). While these changes are intended

to improve their functionalities as food ingredients, such structural changes could also potentially impact their metabolic fate through the gastrointestinal tract. Although acetylation of readily digested native starches is considered to be 1 method by which the content of RS is increased, data from animal and human studies indicate that digestibility of HAMSA is unlikely to be altered by any appreciable extent in comparison to native HAMS, a naturally occurring form of RS (Morita *et al.*, 2005; Bird *et al.*, 2006; Clarke *et al.*, 2007; Ramakrishna, 2012).

Since HAMSA differs from HAMS with respect to the presence of esterified acetyl groups, the main physiological difference anticipated between consumption of HAMSA and HAMS is the increased delivery of acetate to the large intestine. It has been demonstrated using animal models that feeding of diets containing HAMSA (DS 0.18 to 0.2) increases cecal content of total SCFA, particularly acetate, compared to diets containing HAMS (Morita *et al.*, 2005; Bird *et al.*, 2006). In the clinical study conducted by Clarke *et al.* (2007), ingestion of 20 g HAMSA (6% acetylation; DS 0.23) was estimated to provide an additional 18.5 mmol of acetate to the large intestines, when compared to the consumption of an equivalent amount of HAMS.

6.3 Toxicological Evaluation

6.3.1 Authoritative Reviews Conducted for Starch Acetate

The safety of various chemically modified starches, including starch acetate (defined as starch esterified using either acetic anhydride or vinyl acetate, such that a maximum of 2.5% acetyl groups are introduced), has been assessed in a series of evaluations conducted by JECFA (1969, 1972a, 1974, 1982a,b) and the SCOGS (FASEB, 1979). The toxicological data that was reviewed by both JECFA and the SCOGS included several short-term animal studies ranging from 28 days to 13 weeks in duration, an 89-week study in mice, a 2-year study in rats, and a 3-generation reproductive/developmental study (FASEB, 1979; JECFA, 1982a).

Overall, the SCOGS indicated that cecal enlargement and slight diarrhea were observed in some of the studies where high doses (*i.e.*, 50% or higher in the diet, corresponding to ≥ 50 g/kg body weight/day) of starch acetate ($\geq 2\%$ acetylation) were administered (FASEB, 1979). JECFA further noted that for the group of modified starches as a whole, diarrhea and cecal enlargement were observed at dietary intake levels exceeding 10% [approximately 10 g/kg body weight/day (U.S. FDA, 1993)] (JECFA, 1972b). The cecal enlargement was not associated with any histopathological changes (JECFA, 1972b, 1982a; FASEB, 1979). Furthermore, JECFA noted that loose stools and diarrhea are normal physiological consequences of increased bulk in the colon and rectum from the ingestion of high levels of starches (both modified and native), which could lead to functional hypertrophy of the cecum (JECFA, 1972b). As such, these effects were not considered to be toxicologically relevant by both JECFA and the SCOGS (JECFA, 1972b, 1982a; FASEB, 1979).

In the 2-year feeding study in rats, males administered 30% dose (15 g/kg body weight/day) of acetylated potato starch (1.98% acetylation) had a higher frequency of suburothelial deposits of calcium, accompanied by hyperplasia of the epithelium lining the renal pelvis, compared to controls (Til *et al.*, 1971a; de Groot *et al.*, 1974). Additionally, a slightly higher incidence of intratubular nephrocalcinosis and concretions in the renal pelvic space (without hyperplasia) was observed in the male mice administered acetylated potato starch at 55% in the diet (*i.e.*, 80 g/kg body weight/day) compared to controls for 79 weeks (Feron *et al.*, 1978). Animals administered the acetylated starch also had 50 to 100% increase in water consumption compared to controls. JECFA further noted that kidney lesions have been reported in long-term studies of animals administered various modified starches in the diet at 5 to 25% (JECFA, 1982b). However, JECFA indicated that these renal lesions are likely manifestations of mineral imbalance in the diet (*i.e.*, Ca, Mg, P), and that such changes commonly occurs even in untreated laboratory rodents (JECFA, 1982a,b; JECFA, 1982b).

The results of a 3-generation reproductive/developmental study indicate that starch acetate did not adversely affect fertility, litter size, resorption quotient, preweaning mortality or growth rates of the pups (Til *et al.*, 1971b; de Groot *et al.*, 1974). In this study, acetylated potato starch (1.98% acetyl content, degree of substitution 0.079) was administered to groups of 10 male and 20 female rats (species not specified) at 10% in the diet [approximately 5 g/kg body weight/day (U.S. FDA, 1993)] (Til *et al.*, 1971b; de Groot *et al.*, 1974). The animals of the P, F₁, and F₂ generations produced 2 successive litters by mating at Week 12 and at Week 20 after weaning.

JECFA and the SCOGS also evaluated 1 report where 12 human subjects consumed 60 g/day of starch acetate (1.98% acetyl content, source not provided) for 4 consecutive days, without any adverse effects on the frequency or amount of feces, or fecal water and lactic acid content, or any other adverse effects (not further described) (Pieters *et al.*, 1971).

Although the majority of the studies administered acetylated potato starch (mostly 2% acetylation), both JECFA and FASEB considered the findings to be relevant to acetylated starches in general, regardless of the plant source of the native food starch (JECFA, 1982a; FASEB, 1979). Overall, the SCOGS concluded that *“there is no evidence in the available information on starch acetate that demonstrates or suggests reasonable groups to suspect a hazard to the public when it is used at levels that are now current and in the manner now practiced”* (FASEB, 1979). According to the SCOGS, the intake of modified starch by the total U.S. population (age 2 to 65 years) was estimated to be 11.8 g/day using dietary intake data, or approximately 0.7 to 1 g/person/day based on marketing data (see Section 5.1) (FASEB, 1979). Similarly, JECFA concluded the ADI for starch acetate to be “not specified”, meaning that the total daily intake of the ingredient, arising from its use or uses at levels necessary to achieve the desired effect, and from its acceptable background in food, does not represent a hazard to health (JECFA, 1982a,b).

6.3.2 Studies Conducted with HAMSA

6.3.2.1 *Animal Studies*

In an unpublished study, administration of HAMSA (DS 0.32) at 10% in the diet [10 g/kg body weight/day (U.S. FDA, 1993)] for 7 days did not significantly alter the gastrointestinal passage rate of the digesta compared to animals fed a control diet containing the same amount of LAMS (Ramakrishna, 2012). Administration of HAMSA did significantly increase fecal bulk, but resulted in significantly lower moisture content of the digesta, compared to administration of LAMS, suggesting that the modified starch would not promote diarrhea.

In an unpublished 28-day study, rats (species and numbers of animals used not provided) were administered diets containing LAMS, 5 or 10% HAMS [5 or 10 g/kg body weight/day (U.S. FDA, 1993)], or HAMSA (DS not provided) at 5 or 10% [5 or 10 g/kg body weight/day (U.S. FDA, 1993)] (Ramakrishna, 2012). At the end of the study, the animals were terminated and various organs were collected for macroscopic and histopathological examination. Blood was also collected for hematology and serum biochemistry analysis. The animals were not fasted prior to termination, and the contents of the cecum and colon were collected for analysis.

Administration of HAMSA did not result in any significant differences in hematological and serum biochemistry parameters compared to either the LAMS or HAMS control groups. There was a significant increase in the dry matter content, significant increase in free acetate pool, and significant decrease in pH of the cecal digesta in animals administered HAMSA at 10% in the diet, compared to the animals administered 10% HAMS. There were no abnormal pathological findings that could be attributed to the administration of HAMSA, and additional organ-bath studies demonstrated that receptor and non-receptor based contractility of the colonic tissue was not significantly altered by administration of HAMSA.

6.3.2.2 *Clinical Studies*

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).

The majority of the clinical trials involving resistant starches have been conducted using RS₂ (e.g., HAMS) or RS₃ (i.e., retrograded starch) (Grabitske and Slavin, 2009). 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 (RS₂ and RS₃) 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).

The tolerability of HAMSA (6% acetylation) has been investigated in a cross-over study conducted by Ramakrishna (2012) (see also Section 6.2.2.3). Among 15 healthy volunteers, the ingestion of warm flavored drinks containing HAMS or HAMSA (6% acetylation) at levels of 20 or 40 g/day for 2 weeks was well-tolerated, with no adverse events were reported during the study. There were no significant differences in mean body weights over the course of the study, and clinical chemistry parameters were not affected by consumption of the HAMS or HAMSA at either dose. Participants completed a Gastrointestinal Quality of Life Index (GIQLI) questionnaire at baseline and following each 2-week intervention, which includes questions related to the occurrence of abdominal pain, fullness, bloating, passage of gas, burping, frequency of bowel movements, satiety, regurgitation, frequency of urgent bowel movements, incidence of diarrhea, constipation, nausea and heartburn. There were no significant differences in the GIQLI scores among subjects receiving up to 40 g/day of HAMSA, compared to administration of HAMS at the same dose, indicating that HAMSA and HAMS have comparable gastrointestinal acceptability and tolerability.

6.4 Water Absorption and Utility of HAMSA2.5 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.

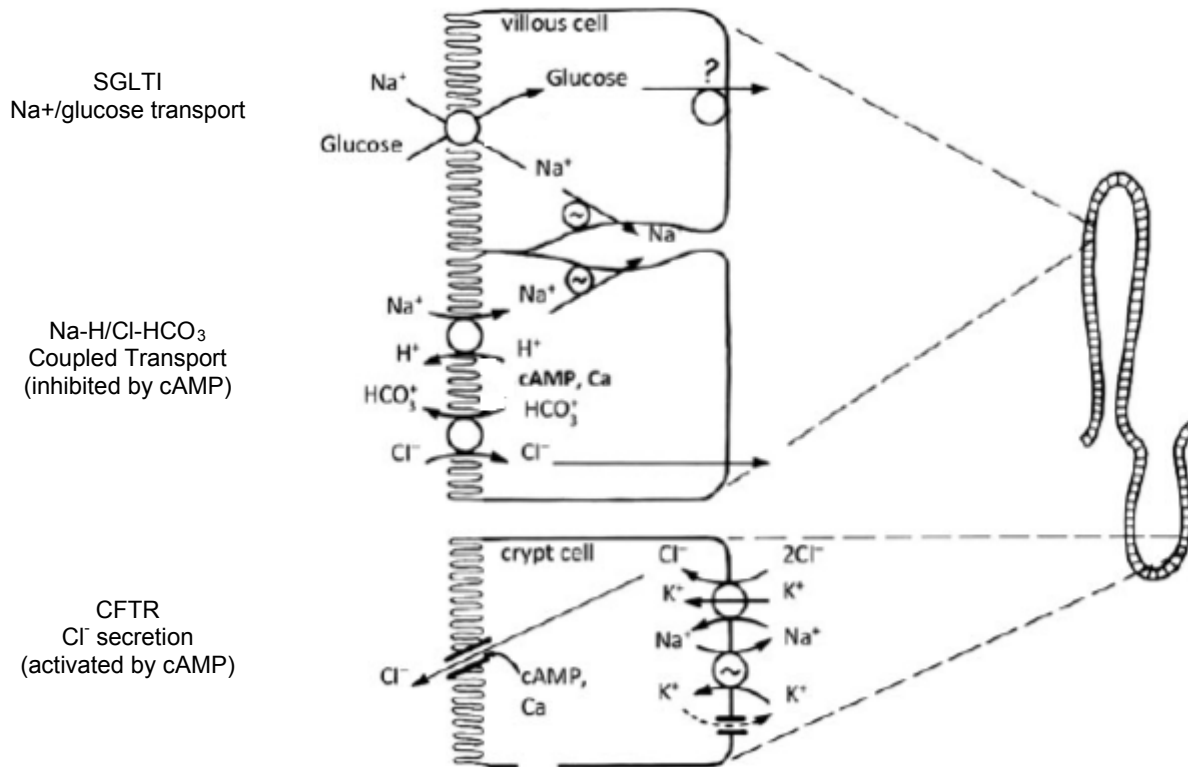
The columnar epithelial monolayer is responsible for electrolyte absorption and secretion within the intestine (Figure 6.4.1-1). Electrolyte secretion occurs mainly in the crypt cells and absorption is largely restricted to the villus or surface epithelium, although some regional overlap of these processes exist (Kopic and Geibel, 2010). Electroneutral absorption of sodium is an important regulator 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 cyclic adenosine monophosphate (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)



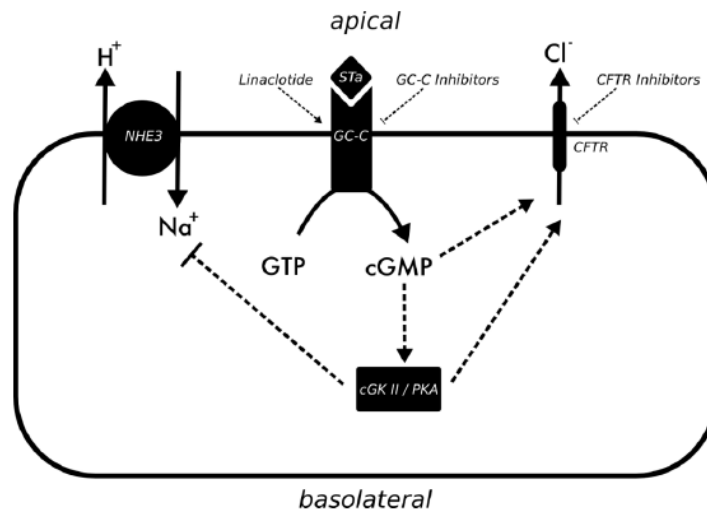
6.4.2 Water Loss during Bacterial and Viral Gastroenteritis

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-HCO₃ co-transporter (NHE3), enterotoxins that produce increases in cAMP levels increase the osmotic pressure in

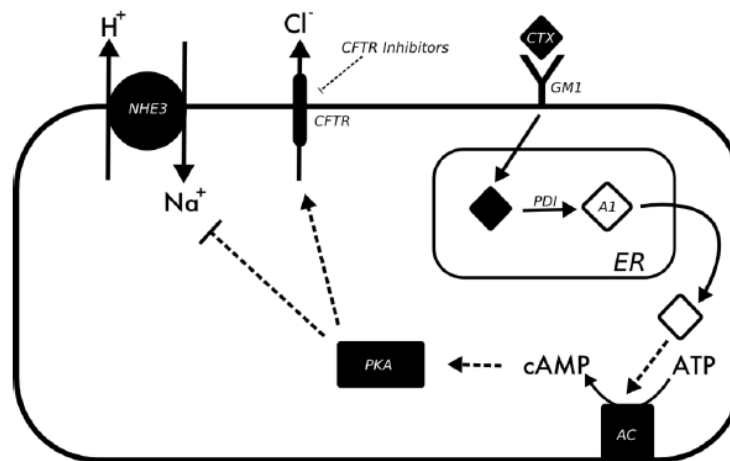
the gut lumen by preventing the absorption of sodium and chloride (Figure 6.4.2-1). The osmotic pressure of the chyme is further increased by the action of enterotoxins as cAMP/cGMP activates the CFTR resulting in the secretion of large quantities of chloride into the gastrointestinal lumen. This dual effect of reduced Na^+ and Cl^- absorption and increased Cl^- secretion caused by dysregulation 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.

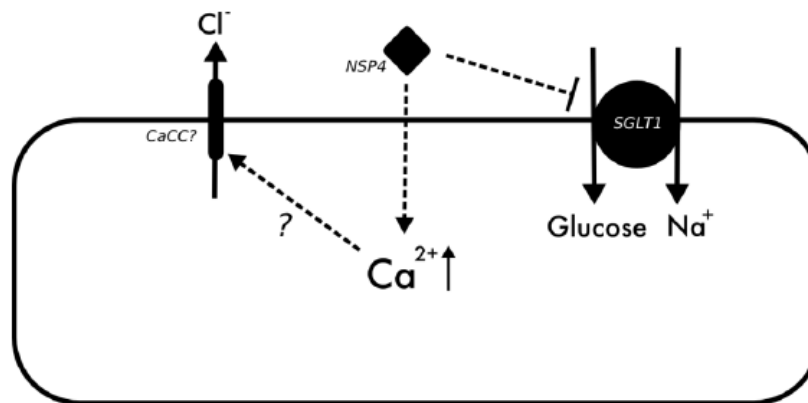


B.



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 SGLT1 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 from 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 osmolality 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 an effective rehydration option for this disease (WHO/ICDDRDB, 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 hydrogen⁵ (from microbial fermentation) in individuals provided a lactulose⁶ 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.

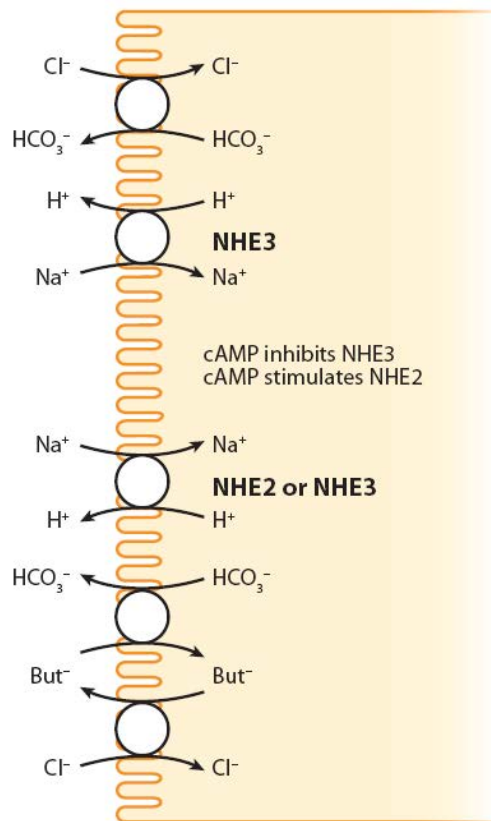
In vitro studies using isolated rat colon mucosa have suggested that significant SCFA absorption occur *via* non-ionic diffusion. It is also recognized that SCFA absorption occurs

⁵ Fermentation of lactulose by gut microflora produce SCFA and hydrogen gas.

⁶ 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.

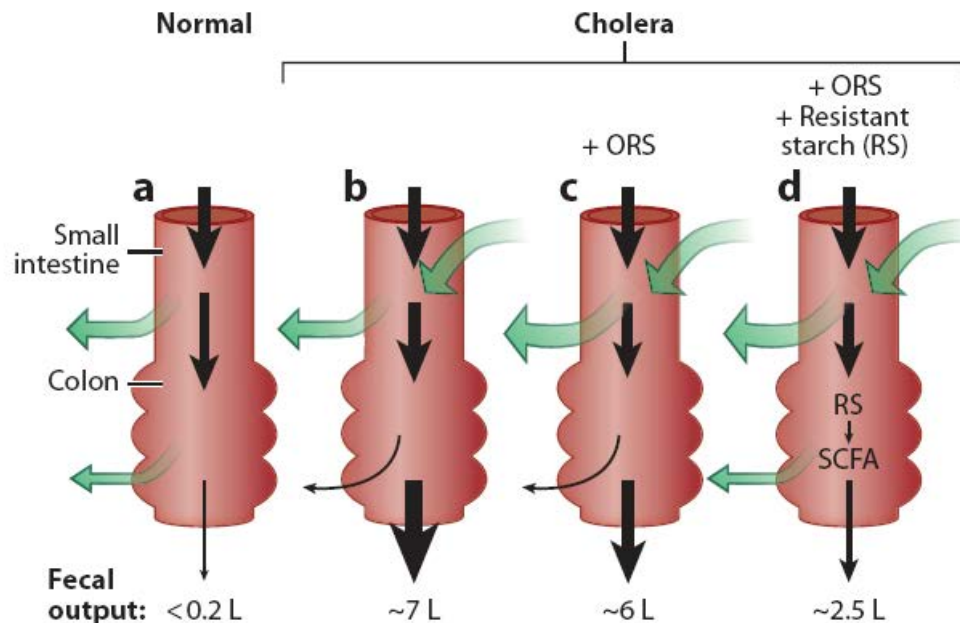
through transport mechanisms; though the specific identity of the SCFA transporters in the human colon remains unknown, experiments conducted using apical membrane vesicles from human ileum and rat distal colon have provided convincing evidence for the existence of a sodium-dependent SCFA-HCO₃ exchange 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 butyrate-HCO₃ exchange that works in parallel with butyrate-Cl transport (Figure 6.4.3-1). This system is similar Na-H/Cl-HCO₃ coupled transport process discussed in Section 6.4.2 above that is inhibited by cAMP. However, unlike the cyclic AMP sensitive Na-H/Cl-HCO₃ exchange, the transport of butyrate through the butyrate-HCO₃ 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 CHO₃-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, specifically HAMSA and HAMS, in treating dehydration of diarrhea are presented below in Section 6.4.4 and 6.4.5, respectively.

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 Studies of HAMSA in Oral Rehydration Solution

6.4.4.1 Animal Studies

Clarke *et al.* (2011) has investigated the effect of HAMSA on the fluid and electrolyte uptake in a whole-gut perfusion model of cholera-toxin induced diarrhea. Perfusion of the gut with a hypotonic ORS containing HAMSA (DS not provided) at 10, 25, or 50 g/L significantly increased net water absorption from the intestines when compared to administration of the same ORS containing either 10 or 50 g/L of HAMS. The net absorption of sodium, chloride, and potassium were also enhanced, while the excretion of bicarbonate was reduced, by administration of HAMSA at 10, 25, or 50 g/L compared to 10 or 50 g/L of HAMS.

6.4.4.2 Human Studies

The effect of ORS solutions containing 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 (2360 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 Studies of HAMS in Oral Rehydration Solution

As discussed in Section 6.2.2, HAMSA and HAMS have comparable extent of digestion in the upper gastrointestinal tract, and thus they are expected to provide similar amounts of fermentable substrates to the colon. Although HAMSA is anticipated to provide a greater amount of acetate in the colon, being released by the action of bacterial lipases and/or esterases and produced from fermentation of the liberated HAMS, the capacity for fermentation of resistant starches by the colonic microflora, as well as the absorption of SCFA in the colon, are maintained even during infectious diarrhea (Binder, 2010; Binder *et al.*, 2014). Therefore, the animal and clinical studies that have investigated the suitability of ORS containing HAMS, the source material of HAMSA2.5, for the management of dehydration during acute diarrhea can also be used to corroborate the safety of HAMSA2.5 in ORS.

6.4.5.1 Animal Studies

Using a whole-gut perfusion model 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 *E. 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 beneficial effect of RS-ORS 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 two-folds; HAMS can be hydrolyzed and stimulate sodium-glucose cotransport 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.

6.4.5.2 Human Studies

A total of 4 studies identified that investigated the effects of ORS containing HAMS, compared to standard glucose-based ORS, in human subjects with diarrhea and diagnosed dehydration (Ramakrishna *et al.*, 2000, 2008; Raghupathy *et al.*, 2006; Alam *et al.*, 2009). The characteristics of these studies are summarized in Table 6.4.5.2-1.

Two of the studies were conducted in young children (less than 5 years of age) (Raghupathy *et al.*, 2006; Alam *et al.*, 2009), while 2 of the studies were in adults (considered as age 12 or older) (Ramakrishna *et al.*, 2000, 2008). 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). In 3 out of the 4 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 up to 500 g HAMS over the course of 48 hours (*i.e.*, approximately up to 125 g/day).

Overall, administration of HAMS, 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 help support 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 reabsorption of water and electrolytes without adding a large osmotic load to bowel contents (Gregorio *et al.*, 2009). It has also been suggested that partially digested carbohydrates may undergo fermentation in the colon, which would also aid in the absorption of fluids and electrolytes (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 maize⁷). 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 also decreased the duration of diarrhea among adults positive for *V. cholerae* compared to

⁷ 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.

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) did not differ 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 would not pose any safety concerns, and would be expected to improve dehydration at least as well as standard glucose-based ORS. 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.

Table 6.4.5.2-1 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
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 h, 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 72 h) • 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 755 mL) • 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 h 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 <i>et al.</i> , 2006
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 h 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 ◦ NSD in body weight gain over 72 h ◦ NSD in stool, vomit, and urine output ◦ NSD in the duration of diarrhea	Alam <i>et al.</i> , 2009

Table 6.4.5.2-1 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
				- 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 <i>et al.</i> , 2009
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>i.e.</i>, 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 <i>et al.</i> , 2000
Randomized, and controlled Single-blinded: Subjects were not blinded due to visible differences in the test articles ^a	50 adult males with watery diarrhea (<3 days) and moderate to severe dehydration (subgroup with cholera, n=22) Ages: 12 to 65 (HAMS-ORS: 42.6±12.0; ORS: 37.8±13.7)	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)	- 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	Ramakrishna <i>et al.</i> , 2008

Table 6.4.5.2-1 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
				diarrhea in patients with or without positive <i>Vibrio</i> cultures	
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>i.v.</i> 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 <i>et al.</i> , 2013 [abstract]

bw = body weight; h = hour; 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

Yale intends to use HAMSA2.5, meeting the specifications for starch acetates (INS No. 1420) established by the FCC and JECFA (JECFA, 2013; FCC, 2014), as a replacement for glucose in hypo-osmolar ORS. Food starches that have been acetylated *via* treatment with acetic anhydride or vinyl acetate, such that the proportion of acetyl groups in the final product does not exceed 2.5% (DS=0.1), is a widely accepted form of modified starch that is used widely in the food industry (FASEB, 1979; JECFA, 1982a, 2013; FCC, 2014). HAMSA2.5 specifically is the acetylated form of HAMS containing 2.5% acetyl group; HAMS is derived from maize that has been developed to contain high amylose content through traditional breeding methods, and is a form of starch that is resistant to digestion in the upper gastrointestinal tract. Commercially available forms of HAMS have been available for use as a food ingredient since the 1950s (BeMiller, 2009). As such, both HAMSA2.5 and HAMS have a long history of safe consumption in the diet.

Similarly to other dietary fibers, the primary safety concern associated with the ingestion of resistant starches such as HAMSA2.5 is the potential for adverse gastrointestinal effects, particularly at high doses. Resistant starches such as HAMSA2.5 and HAMS are incompletely digested in the upper gastrointestinal tract and instead undergo fermentation by the colonic microflora, which results in the production of gases and SCFA (Brown, 1994; Topping and Clifton, 2001; Binder, 2010). Ingestion of resistant starches at doses that exceed the colonic capacity for fermentation, and/or the capacity of SCFA absorption, could potentially result in osmotic diarrhea. In animal studies, cecal enlargement and diarrhea has been observed following ingestion of large doses (≥ 50 g/kg body weight/day) of starch acetate ($\geq 2\%$ acetylation) (FASEB, 1979; JECFA, 1982a). However, in the absence of histopathological changes, and the fact that increased bulk of cecal contents are considered a normal physiological response to ingestion of high levels of starches, JECFA and the SCOGS did not consider these effects to be toxicologically relevant (JECFA, 1972b, 1982a; FASEB, 1979). In humans, the acceptable daily intakes of resistant starches (RS₂ and RS₃) from various sources has been determined to be approximately 45 g/day, based on the increased incidence of excessive flatulence reported at higher doses (Grabitske and Slavin, 2009). Furthermore, the fermentability of resistant starches is generally thought to be complete, at least in healthy individuals at levels of intake that are typically observed in the diet, and they appear to have a high laxation threshold compared to other dietary fibers, with only rare cases of diarrhea reported even at doses as high as 80 g/day (Grabitske and Slavin, 2009).

Depending on the extent of dehydration, large volumes of ORS may be consumed to replace the existing and ongoing fluid losses from acute diarrhea. Based on the recommendations of authoritative bodies, the amount of ORS consumed could be as high as 6 L/day for adults. As HAMSA2.5 will be added to ORS at 50 g/L, the amount of HAMSA2.5 consumed could be as

high as 300 g/day. Nevertheless, it is important to note that the recommended volumes of ORS to be consumed apply to diarrhea of all etiologies, including cholera diarrhea where fluid and electrolyte losses are high. It is not anticipated that individuals with acute diarrhea, with or without mild to moderate dehydration in the U.S., will consume such large volumes of ORS and large amounts of HAMSA2.5. Moreover, human trials have demonstrated that the intake of HAMSA in ORS would not further exacerbate diarrhea. No adverse events were observed when ORS containing 50 g/L of HAMSA was administered to 100 adults with acute diarrhea (24% with cholera diarrhea), and moderate to severe dehydration (Pal *et al.*, 2013). Furthermore, subjects consuming the ORS containing HAMSA had significantly shorter duration of diarrhea, and fecal weight did not significantly differ, when compared to subjects consuming an ORS containing 50 g/L of HAMS. Therefore, administration of ORS containing 50 g/L HAMSA does not further exacerbate diarrhea or result in adverse gastrointestinal effects, even when large volumes are consumed.

As additional support for the safety of HAMSA2.5 when used as a carbohydrate source in ORS, clinical studies where ORS containing similar levels of HAMS (50 g/L) as the intended uses of HAMSA2.5, have also been without adverse effects. For example, administration of a hypo-osmolar ORS where glucose was substituted by 50 g/L of HAMS, there were no adverse effects in terms of the need for unscheduled intravenous fluid therapy or dialysis, and serum electrolyte levels were not significantly different compared to subjects receiving the standard glucose-based hypo-osmolar ORS (Ramakrishna *et al.*, 2008). Furthermore, administration of ORS containing HAMS significantly reduced the fecal weight during the first 48 hours, and the duration of diarrhea, compared to administration of the standard glucose-based ORS. Thus, these results suggest HAMS is a suitable replacement for glucose in ORS used in the management of acute diarrhea.

7.0 CONCLUSION

Based on available data and information presented herein, Yale has determined that acetylated (2.5%) 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) under the intended conditions of use as a replacement for glucose in oral rehydration solutions that are medical foods, based on scientific procedures.

8.0 REFERENCES

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

May 25, 2015

Yale University (Yale) intends to market Acetylated (2.5%) High-Amylose Maize Starch (HAMSA2.5) as an nutrient ingredient 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 relevant national and international experience and scientific training 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 intended uses of High-Amylose Maize Starch (HAMS) as nutrient source of glucose in ORS is safe 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). These Panel members have been determined to be qualified by relevant experience and scientific training to evaluate the safety of HAMS under the aforementioned food use.

The Expert Panel, independently and collectively, critically evaluated a dossier¹ which included details pertaining to the method of manufacture and product specifications, supporting analytical data, intended use conditions in ORS, estimated exposure under the intended uses, and a comprehensive assessment of the available scientific literature pertaining to the safety of the intended uses of HAMSA2.5. The Expert Panel also evaluated other information deemed appropriate or necessary. Following 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 intended uses described herein for HAMSA2.5, meeting appropriate food-grade specifications as described in the supporting dossier and manufactured according to current Good Manufacturing Practice (cGMP), are GRAS based on scientific procedures. A summary of the basis for the Expert Panel's conclusion is provided below.

¹ Documentation Supporting the Determination that High-Amylose Maize Starch Acetate (HAMSA2.5) 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 acetylated (2.5%) high-amylose maize starch (HAMSA2.5). HAMSA2.5 meets the definition of starch acetate (INS No. 1420), which is esterified *via* treatment with acetic anhydride or vinyl acetate such that acetyl groups account for no more than 2.5% in the final product. The material meets the specifications for modified food starches as defined in the Food Chemical Codex (FCC, 2014) and in the monograph issued by Joint FAO/WHO Expert Committee on Food Additives (JECFA) (JECFA, 2013).

In the United States (U.S.), ORS can be considered as a form of nutritional support during episodes of diarrhea, and they are classified as medical foods (U.S. FDA, 2006). 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 HAMSA2.5 or other types of chemically modified starches being used as a carbohydrate source in ORS in the U.S., though rice-based ORS (*e.g.*, CeraLyte[®]; Ricelyte[®]) are currently marketed.

HAMSA2.5 is intended for use as a nutrient 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 (>2 years of age). HAMSA2.5 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 HAMSA2.5 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 Prevention² (CDC) (CDC, 2003; WHO, 2005; WGO, 2012). According to these guidelines, oral rehydration therapy typically consists of 2 phases. 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 of level of 50 g/L of HAMSA2.5 in ORS could result in theoretical maximum intake of 105 to 250 g HAMSA2.5 among children (>2 years) and

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).

teenagers (up to 15 years), and up to 300 g HAMSA2.5 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), the intake of ORS is expected to be less. 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. 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 HAMSA2.5), and adults consume approximately 2 L/day (providing 100 g/day of HAMSA2.5), to replace ongoing fluid losses from each loose stool or vomiting episode (WHO, 2004, 2005). Moreover, exposures to HAMSA2.5 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). As acetylated high-amylose maize starch (HAMSA) and other resistant and chemically modified 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 to be trivial to intakes from ORS uses, and moreover, as gastroenteritis is typically associated with reduced feeding additive consumption of resistant starches from the background diet would likely be minimal during ORS consumption.

Native food starches (e.g., high-amylose maize starch) that have been chemically modified to contain 2.5% acetyl groups (*i.e.*, starch acetate, INS No. 1420) is a food additive that has a long history of safe consumption by populations globally. In the U.S., the Select Committee on GRAS Substances (SCOGS) has evaluated the safety of various types of modified starches, including starch acetate (FASEB, 1979). It was concluded that *“there is no evidence in the available information on starch acetate that demonstrates or suggests reasonable grounds to suspect a hazard to the public when it is used at levels that are now current and in the manner now practiced”* (FASEB, 1979). As such, starch acetate is a permitted direct food additive that may be used without any limitations aside from adherence to Good Manufacturing Practice in the U.S. (21 CFR § 172.892 - U.S. FDA, 2013). Starch acetate is also a permitted food additive without any restrictions on its use in many other jurisdictions. JECFA has established an Adequate Daily Intake level for starch acetate 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, 1982a,b). Accordingly, starch acetate is listed in the Codex General Standard for Food Additives as being permitted to be added to foods in general at *quantum satis* levels (CODEX, 2013).

Acetylated high-amylose maize starch is considered to be a type of resistant starch (RS₂, RS₄) as its digestion within the small intestines is incomplete, and a fraction of the ingested dose will reach the large intestines 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) has 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 fiber³, including resistant starches such as HAMSA2.5, 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 Intake⁴ 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).

In general, starch digestion is mediated mainly through the actions of pancreatic α -amylase in the small intestines, 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 is relatively low during the first few months of life, as enzyme levels do not reach those of adults until nearly 2 years of age (McClellan and Weaver, 1993). Acetylation of readily digested native food starches generally increases the resistant starch content, but acetylation of HAMS, which is a naturally occurring form of resistant starch (RS₂), does not appear to have a large impact on its digestibility. *In vitro*, the digestibility of HAMSA (3.35 to 3.83% acetylation) was actually 24 to 26% higher than the unmodified form of native starch (Liu *et al.*, 1997). In colectomized rats, the digestibility of HAMSA with 4.8% acetylation was significantly decreased (at 47.5%) compared to the digestibility of unmodified HAMS (at 58%), and both were appreciably less digestible than low-amylose waxy maize starch where digestion was virtually complete at 99.8% (Morita *et al.*, 2005). Although the fecal excretion of undigested starch was significantly increased in rats fed a fiber-free diet containing HAMSA (4.8% acetylation) at 200 g/kg diet [*i.e.*, 20 g/kg body weight/day (U.S. FDA, 1993)],

³ The IOM considers resistant starch that is naturally occurring 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).

⁴ 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).

compared to animals fed an equivalent amount of native HAMS for 14 days, it was unclear whether these findings were due to the fact that the animals were fed a restricted fiber free diet, which may have reduced the diversity of the microflora, or to some specific attribute of HAMSA or HAMS (Morita *et al.*, 2005). Furthermore, in rats administered diets containing 15% [*i.e.*, 15 g/kg body weight/day (U.S. FDA, 1993)] of industrially or laboratory synthesized HAMSA (DS 0.2), in a background diet containing 40% HAMS for 7 days, the wet weight of the fecal content did not significantly differ when compared to animals fed diets containing only 55% HAMS (Bird *et al.*, 2006). Among ileostomists (n=7) who consumed custards containing 20 g of cooked and cooled starches for 3 days, the ileal digestibility of HAMSA (6% acetylation; DS 0.23) was reported at 71.6%, which was not significantly different from the digestibility of HAMS at 72.9% (Clarke *et al.*, 2007). Acetylation of HAMS has minimal effect on the fermentation efficiency within the colon. For example, in a group of 15 healthy volunteers consuming warm drinks containing 20 or 40 g of raw HAMS or HAMSA (providing 20 or 40 mmol acetate respectively) daily for 2 weeks, the overall digestibility of HAMSA, as determined by fecal measures of starch content, was reduced by <5% relative to HAMS.

Toxicological studies pertaining to the safety of various chemically modified starches, including starch acetate, has been evaluated by authoritative bodies including the SCOGS (FASEB, 1979) and JECFA (JECFA, 1982a). These include several short-term animal studies ranging from 28 days to 13 weeks, an 89-week study in mice, a 2-year study in rats, and a 3-generation reproductive/developmental study in rats. The SCOGS noted that cecal enlargement and slight diarrhea were observed in animals administered diet containing high doses (*i.e.*, 50% or higher, corresponding to ≥ 50 g/kg body weight/day) of starch acetate with $\geq 2\%$ acetylation (FASEB, 1979). JECFA further noted that these effects were observed at dietary doses of 10% or higher [*i.e.*, ≥ 10 g/kg body weight/day (U.S. FDA, 1993)] for modified starches as a whole (JECFA, 1972). Nevertheless, loose stool and cecal enlargement is considered to be a physiological response to the ingestion of large amount of starches, and given the lack of associated histopathological changes, these findings were not considered to be toxicologically relevant by JECFA and the SCOGS (JECFA, 1972; FASEB, 1979; JECFA, 1982a).

In long-term studies, kidney lesions were also reported following the dietary administration of starch acetate. In a 2-year feeding study, rats (30/sex/group) were administered diets containing acetylated potato starch (1.98% acetylation) at 0, 5, 10, and 30% (corresponding to 0, 2.5, 5, and 15 g/kg body weight/day) (Til *et al.*, 1971a; de Groot *et al.*, 1974). Males administered the 15 g/kg body weight/day dose had a slightly increased incidence of nephrocalcinosis, accompanied by focal hyperplasia of the pelvic epithelium, compared to the control animals (*i.e.*, 3 “slight” and 1 “moderate” lesions in the starch acetate group, and 2 “slight” and 1 “moderate” lesions in the control). Additionally, a significant increase in the incidence of intratubular calcareous deposits, as well as slightly calcareous deposits in the renal pelvis and increased incidence of thickened urinary bladder epithelium (without hyperplasia) were observed in Albino SPF Swiss Random mice (75/sex/group) administered acetylated

potato starch (1.98% acetylation) at 55% in the diet (approximately 80 g/kg body weight/day) for 89 weeks, compared to control animals administered the same amount of pregelatinized potato starch (Feron *et al.*, 1978). Although renal lesions have also been observed in animals administered other forms of modified starch, they are considered to be due to the mineral imbalance in the diet (Ca, Mg, and P), and such lesions are commonly even among untreated laboratory rodents; thus, they were not considered to be toxicologically relevant (FASEB, 1979; JECFA, 1982a,b). No reproductive/developmental toxicity (*i.e.*, changes in fertility, litter size, resorption quotient, preweaning mortality or growth rates of the pups) was observed in a 3-generation study in rats administered acetylated potato starch at 10% in the diet [corresponding to approximately 5 g/kg body weight/day (U.S. FDA, 1993)] (Til *et al.*, 1971b; de Groot *et al.*, 1974).

In humans, ingestion of high doses of resistant starches is associated with undesirable gastrointestinal side effects such as abdominal discomforts, bloating, and flatulence (Livesey, 2001; Grabitske and Slavin, 2009). Following a systematic review of the literature pertaining to the gastrointestinal side effects of resistant starch, Grabitske and Slavin (2009) estimated the acceptable daily intakes of resistant starches (RS₂ and RS₃) 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). The tolerability of large intakes of HAMS and HAMSA in healthy individuals was evaluated by Ramakrishna (2012). Using a randomized cross-over design, a group of 15 healthy subjects consumed warm drinks containing HAMS or HAMSA (6% acetylation) at levels providing acute daily intakes of 20 or 40 g/day for 2 weeks. Each treatment period included two 48-hour fecal collections, and measures of pH, fecal output, moisture content, ammonia and starch content were obtained. All participants were instructed to complete the Gastrointestinal Quality of Life Index (GIQLI)⁵ as a measure of acceptability and potential side-effects. The body weights of the subjects remained stable throughout the study and all of the treatment intervals were well-tolerated and did not result in undesirable gastrointestinal side-effects. No statistically significant differences in fecal pH, fecal output, fecal moisture content or fecal ammonia were observed. There also were no statistically significant differences in fecal concentrations of short-chain fatty acids (SCFA). Findings from this study demonstrate that the large intestine has a high capacity for metabolism of HAMS/HAMSA, and that large quantities of HAMS/HAMSA (*i.e.*, levels $\geq$ than that provided in 500 ml of ORS) consumed acutely in the diet over multiple days does not saturate the SCFA transporters resulting in diarrhea or other adverse gastrointestinal effects. Similar findings that acetylated starches are well tolerated are reported in the SCOGS and JECFA evaluations, which reference one study where 12 healthy subjects consumed 60 g/day of acetylated waxy starch (1.98% acetyl content) for 4 days (Pieters *et al.*, 1971). Although it was not mentioned

⁵ GIQLI questionnaire include questions relating to abdominal pain, fullness, bloating, passage of gas, burping, frequency of bowel movement, incidences of diarrhea, constipation, nausea and heartburn.

whether a control group was included in the study, it was noted that there were no adverse effects observed with respect to the frequency or amount of feces, fecal water and lactic acid content, or any other adverse effects (Pieters *et al.*, 1971). As acetylated waxy starch is expected to be largely digested in the small intestine with limited transport of undigested material to the colon, this study demonstrates that provision of acetate to the small intestine from consumption of starch acetate also is well tolerated.

Under normal circumstances, approximately 8 to 9 L of fluids are absorbed each day 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 disrupts the transport processes in 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-HCO₃ 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 large amounts of water loss (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.*, HAMSA2.5) as a source of glucose in ORS would provide a continuous supply of glucose as they are slowly 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 HAMSA2.5 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-HCO₃ exchanger (Binder and Ramakrishna, 2004; Binder, 2010; Binder *et al.*, 2014).

Multiple studies evaluating the safety and suitability of using chemically modified and resistant maize starch in ORS products intended for rehydration in children and adults with acute gastroenteritis have been reported (Ramakrishna *et al.*, 2000; Raghupathy *et al.*, 2006; Ramakrishna *et al.*, 2008; Alam *et al.*, 2009; Pal *et al.*, 2013). In a randomized, double-blind, controlled study, the use of HAMSA in ORS was evaluated in adult subjects with cholera and non-cholera gastroenteritis (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. 100 adult patients were enrolled, 49 received HAMSA and 51 received HAMS. There were no adverse events attributable to HAMS or HAMSA identified 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 ($p=0.036$).

Findings from controlled studies conducted in ileostomy patients and healthy subjects discussed above demonstrate that the use of HAMS or HAMSA in ORS will provide similar quantities of digestible glucose to the small intestine, differences in metabolism of HAMS vs. HAMSA are limited to provision of additional acetate to the colon following consumption of HAMSA. HAMSA intended for use in ORS will be compliant with FCC specifications and therefore will contain up to 2.5% acetate on a wt/wt basis. The potential consumption of 500 ml of ORS containing HAMSA at a use level of 50 g/L would result in the delivery of 10 mmol of additional acetate to the large intestine available for facilitation of water absorption. The large intestine has a high capacity for SCFA absorption (Holtug *et al.*, 1992), and controlled tolerance studies in healthy subjects administered HAMSA have demonstrated that acetylation levels up to 6% do not saturate SCFA absorption pathways (Ramakrishna, 2012). Both HAMS and HAMSA therefore have similar digestive fates: 1) Both deliver a readily digestible source of glucose to the small intestine; 2) Both starch products provide significant concentrations of SCFA to the small intestine. Accordingly, studies evaluating the use of HAMS in ORS applications in adults and children with acute gastroenteritis were considered applicable to the safety assessment of HAMSA.

Findings from 4 published clinical studies evaluating the use of HAMS based ORS in adults and children with acute diarrhea were therefore reviewed by the Expert Panel. 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 (HAMS-ORS). The age (mean±SD) of the participants was 37.8±13.7 years in the HO-ORS group and 42.6±12.0 years in the HAMS-ORS group, and 22 of the participants tested positive for *Vibrio cholerae*. 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”. Overall, 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 of the 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 for longer than 48 hours, except in one subject in the HO-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, suggesting that HAMS-ORS does not further exacerbate the diarrhea.

In other randomized and controlled studies, ORS were administered that contained HAMS as an adjunct to glucose, rather than being a complete replacement. In 48 adults (age 14 to 58) with cholera diarrhea (<72 hours in duration), administration of the WHO-endorsed iso-osmolar (~327 mOsm/kg) glucose-based ORS containing additional 50 g/L of HAMS did not exacerbate diarrhea, as indicated by the fecal weight or time to form first solid stool, when compared to administration of the same ORS without glucose (Ramakrishna *et al.*, 2000). The addition of HAMS at 50 g/L as an adjunct to glucose in standard WHO-endorsed iso-osmolar ORS was also without adverse effects among young children (ages 6 months to 5 years), such as increased vomiting or exacerbation of diarrhea, increased need for unscheduled intravenous fluid therapy, mortality, or changes in serum electrolyte levels (Raghupathy *et al.*, 2006; Alam *et al.*, 2009).

Overall, findings from multiple randomized controlled studies in children and adults are sufficient to 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. Factors 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. Moreover, the Expert Panel 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 of 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). Data from ileostomy patients and *in vivo* dietary studies in pigs suggest that approximately 30 to 40% of the ingested HAMS will be transported to the large intestine. Larger quantities of HAMS/HAMSA may be delivered to the colon during cases of acute gastroenteritis where gastrointestinal transit rates are often reduced. Resistant starch entering the large intestine will be subjected to microbial fermentation resulting in the production of large concentrations of SCFA. Transportation of SCFA across the colonocyte promotes facilitated diffusion of water improving rehydration, and also can promote the co-transport of sodium. 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). The potential effect of HAMS based ORS on sodium status was considered. Controlled studies conducted with HAMS and other resistant starch based ORS formulations (*i.e.*, rice-based ORS) have not identified increased incidences of hypernatremia with these formulations during rotavirus infection (Gregorio *et al.*, 2009). 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) (ESPGAN, 1992). The Expert Panel concluded that the totality of evidence demonstrate that replacement of glucose by 50 g/L HAMS in ORS would not pose any safety concerns among children (>2 years) or adults with acute diarrhea, with or without mild to moderate dehydration, within the United States.

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 intended uses of Acetylated (2.5%) High-Amylose Maize Starch. We unanimously conclude that Acetylated (2.5%) 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 Acetylated (2.5%) 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.

Joseph F. Borzelleca, Ph.D.
Professor Emeritus
Virginia Commonwealth University School of Medicine

Date

George C. Fahey, Ph.D.
Professor Emeritus
University of Illinois

Date

John Doull, M.D., Ph.D.
Professor Emeritus
The University of Kansas Medical Center

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(b) (6)

Joseph F. Borzelleca, Ph.D.
Professor Emeritus
Virginia Commonwealth University School of Medicine

29 September 2015
Date

(b) (6)

George C. Fahey, Ph.D.
Professor Emeritus
University of Illinois

9/30/15
Date

(b) (6)

John Doull, M.D., Ph.D.
Professor Emeritus
The University of Kansas Medical Center

9/28/2015
Date

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