

Index to U.S. Food and Drug Administration's (FDA) Response to Natural Resources Defense Council's (NRDC) October 11, 2013 Freedom of Information Act (FOIA) Request (FDA FOA Request No 2013-8042) for 20 Selected Generally Recognized as Safe (GRAS) Notices (GRN) Submitted to the Agency between 1998 and 2013.

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<sup>1</sup> Posted at <a href="http://www.accessdata.fda.gov/scripts/fcn/fcnNavigation.cfm?rpt=grasListing">www.accessdata.fda.gov/scripts/fcn/fcnNavigation.cfm?rpt=grasListing</a> . <sup>2</sup> See file labelled "chemicals-in-food-FOIA-Main.pdf" for information on other GRNs.				



NATURAL RESOURCES DEFENSE COUNCIL

October 11, 2013

**VIA FAX AND CERTIFIED MAIL**

Food and Drug Administration  
Division of Freedom of Information  
Office of Shared Services  
Office of Public Information and Library Services  
12420 Parklawn Drive  
ELEM-1029  
Rockville, MD 20857  
FAX: (301) 827-9267

**Re: FOIA Request for Records Regarding Generally Recognized as Safe Notices Received by the Agency**

Dear FOIA Officer:

I write on behalf of the Natural Resources Defense Council (NRDC) to request disclosure of records pursuant to the Freedom of Information Act ("FOIA"), 5 U.S.C. § 552, and applicable Food and Drug Administration ("FDA") regulations, 21 C.F.R. Part 20.

**I. Description of Records Sought**

Please produce records<sup>1</sup> in FDA's possession, custody or control on or before September 30, 2013 associated with the Generally Recognized as Safe (GRAS) notices described in Appendix A. We are specifically seeking the following records:

- (a) Communications between FDA and the individual or firm that submitted the GRAS notice to the agency as well as the additive manufacturer or their representative;
- (b) Comments received from other persons outside the agency regarding the GRAS notice;
- (c) Communications between FDA and the European Food Safety Authority regarding the substance or substances described in the GRAS notice; and

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<sup>1</sup> "Records" means anything denoted by the use of that word or its singular form in the text of FOIA and includes correspondence, minutes of meetings, memoranda, notes, emails, notices, facsimiles, charts, tables, presentations, orders, filings, and other writings (handwritten, typed, electronic, or otherwise produced, reproduced, or stored). This request seeks responsive records in the custody of any FDA office, including, but not limited to, FDA Headquarters offices, and specifically including FDA offices in possession of records regarding the GRAS notifications described in Appendix A.



- (d) Memo from FDA's scientific staff describing the preliminary or final results of their evaluation of the GRAS notices exposure assessment, toxicity assessment, safety assessment, or environmental impact.

We are not seeking:

- (a) Copies of the notices and agency decisions FDA posted at <http://www.accessdata.fda.gov/scripts/fcn/fcnNavigation.cfm?rpt=grasListing> as of October 10, 2013.

## **II. Request for a Fee Waiver**

NRDC requests that FDA waive the fee that it would otherwise charge for search and production of the records described above. FOIA dictates that requested records be provided without charge "if disclosure of the information is in the public interest because it is likely to contribute significantly to public understanding of the operations or activities of the government and is not primarily in the commercial interest of the requester." 5 U.S.C. § 552(a)(4)(A)(iii); *see also* 21 C.F.R. § 20.46. The requested disclosure would meet both of these requirements. In addition, NRDC qualifies as "a representative of the news media" entitled to a reduction of fees under FOIA. 5 U.S.C. § 552(a)(4)(A)(ii)(II).

### **A. NRDC Satisfies the First Fee Waiver Requirement**

The disclosure requested here would be "likely to contribute significantly to public understanding of the operations or activities of the government." 5 U.S.C. § 552(a)(4)(A)(iii); 21 C.F.R. § 20.46(a)(1). Each of the four factors used by FDA to evaluate the first fee waiver requirement indicates that a fee waiver is appropriate for this request. *See* 21 C.F.R. § 20.46(b).

#### ***1. Subject of the request***

The records requested here were either received by the agency or generated by the agency as it evaluated the GRAS notice. The requested records thus directly concern "the Government's operations or activities." 21 C.F.R. § 20.46(b).

#### ***2. Informative value of the information to be disclosed***

The requested records are "likely to contribute significantly to public understanding of operations and activities of the Government." 21 C.F.R. § 20.46(a)(1). The public does not currently possess comprehensive information regarding the government's role in addressing public health issues related to the potential use of the listed GRAS substances in food.

We believe that the records requested are not currently in the public domain. Their disclosure would thus meaningfully inform public understanding with respect to food safety, as further discussed below. However, if FDA were to conclude that some of the requested records are publicly available, NRDC would like to discuss that conclusion and might agree to exclude such records from this request.

3. ***Contribution to an understanding of the subject by the public is likely to result from disclosure.***

Because NRDC is a “representative of the news media,” as explained in Part II.C below, FDA must presume that this disclosure is likely to contribute to public understanding of its subject. 21 C.F.R. § 20.45(a)(2).

However, even if NRDC were not a media requester, NRDC’s expertise in food safety, extensive communications capabilities, and proven history of dissemination of information of public interest—including information obtained from FOIA records requests—indicate that NRDC has the ability and will to use disclosed records to reach a broad audience of interested persons with any relevant and newsworthy information the records reveal.

NRDC intends to disseminate any newsworthy information in the released records and its analysis of such records to its member base and to the broader public, through one or more of the many communications channels referenced below. NRDC frequently disseminates newsworthy information to the public for free, and does not intend to resell the information requested here. NRDC’s more than one million members and online activists are a broad audience of persons interested in the subject of GRAS notices, and when combined with NRDC’s communications to the public at large, the likely audience of interested persons to be reached is certainly reasonably broad. As NRDC’s long history of incorporating information obtained through FOIA into reports, articles and other communications illustrates, NRDC is well prepared to convey to the public any relevant information it obtains through this records request.

NRDC has the ability to disseminate information on GRAS notices through many channels. As of September 2013, these include, but are not limited to the following:

- NRDC’s website, available at <http://www.nrdc.org>, which is updated daily and draws approximately 1,142,700 page views and 478,000 unique visitors per month.
- *OnEarth* magazine, which is distributed to approximately 130,000 subscribers, for sale at newsstands and bookstores, and available free of charge at <http://www.onearth.org> (a site that itself has about 33,700 email subscribers and receives more than 45,600 unique visitors per month).
- *Nature’s Voice* newsletter on current environmental issues, which is distributed five times a year to NRDC’s more than one million members and online activists, and is available online at <http://www.nrdc.org/naturesvoice/default.asp>.
- *Earth Action* email list which has more than 179,000 subscribers who receive biweekly information on urgent environmental issues. This information is also made available through NRDC’s online Action Center at <http://www.nrdc.org/action/default.asp>.
- *This Green Life*, which is an electronic newsletter on environmentally sustainable living. It is distributed by email to 52,000 subscribers and made available online at <http://www.nrdc.org/thisgreenlife/default.asp>.
- *NRDC Online*, which is a semimonthly electronic environmental newsletter distributed by e-mail to more than 50,400 subscribers, at <http://www.nrdc.org/newsletter>.
- “Switchboard,” available at <http://switchboard.nrdc.org>, which is a staff blogging site that is updated daily and features more than 130 bloggers writing about current environmental issues. The blogs draw approximately 175,00 page views and 109,200 unique visitors per month; Switchboard’s RSS feeds have approximately 7,500 subscribers; and Switchboard posts appear on websites of other major internet media outlets, such as “The Huffington Post,” at <http://www.huffingtonpost.com>.

- NRDC's profiles on "Facebook," at <http://www.facebook.com/nrdc.org>, and "Twitter," at <http://www.twitter.com/nrdc>, are updated daily and have approximately 210,000 fans and 105,900 followers, respectively.

NRDC issues press releases, issue papers, and reports; directs and produces movies, such as *Stories from the Gulf*, narrated by Robert Redford and *Acid Test*, narrated by Sigourney Weaver; participates in press conferences and interviews with reporters and editorial writers; and has approximately thirty staff members dedicated to communications work, *see* list of select communications staff at <http://www.nrdc.org/about/staff.asp>.

NRDC employees provide Congressional testimony; appear on television, radio and web broadcasts and at conferences; and contribute to numerous national newspapers, magazines, academic journals, other periodicals, and books.

NRDC routinely uses FOIA to obtain information from federal agencies that NRDC legal and scientific experts analyze in order to inform the public about a variety of issues, including energy policy, climate change, wildlife protection, nuclear weapons, pesticides, drinking water safety, and air quality. Some specific examples are provided below:

- (1) In October 2008, NRDC issued a report assessing the degree of enforcement of California's environmental and public health laws. This report, *An Uneven Shield: The Record of Enforcement and Violations Under California's Environmental, Health, and Workplace Safety Laws*, examined data on known violations and law enforcement responses under six critical pollution, health, and workplace safety programs. Much of the data analyzed in the study was obtained through formal FOIA requests; some of it was synthesized from other sources. *See id.* at pp. 4, 16.
- (2) NRDC obtained, through a court-enforced FOIA request, records of the operations of the Bush administration's Energy Task Force, headed by Vice President Dick Cheney. It made those records available, along with analysis of selected excerpts and links to the administration's index of withheld documents, on NRDC's website at <http://www.nrdc.org/air/energy/taskforce/tfinx.asp>. NRDC's efforts helped to inform the public about an issue that, even before the records' release, had attracted considerable attention. *See, e.g.*, Elizabeth Shogren, "Bush Gets One-Two Punch on Energy," *L.A. Times*, Mar. 28, 2002, at A22; Bennett Roth, "Houston Energy-Drilling Firm Appears in Documents from Energy Department," *Houston Chronicle*, Apr. 12, 2002.
- (3) NRDC obtained, through a FOIA request, a memorandum by ExxonMobil advocating the replacement of a highly respected atmospheric scientist, Dr. Robert Watson, as the head of the Intergovernmental Panel on Climate Change. NRDC used this memorandum to help inform the public about what may have been behind the decision by the Bush administration to replace Dr. Watson. *See* NRDC Press Release and attached Exxon memorandum, "Confidential Papers Show Exxon Hand in White House Move to Oust Top Scientist from International Global Warming Panel," Apr. 3, 2002; Elizabeth Shogren, "Charges Fly Over Science Panel Pick," *L.A. Times*, Apr. 4, 2002, at A19.
- (4) NRDC incorporated information obtained through FOIA into a 2005 report, published and provided free of charge at NRDC's website, *see* <http://www.nrdc.org/wildlife/marine/sound/contents.asp>, on the impacts of military

sonar and other industrial noise pollution on marine life. *See Sounding the Depths II: The Rising Toll of Sonar, Shipping and Industrial Ocean Noise on Marine Life* (Nov. 2005) (update to 1999 report). The report also relied upon and synthesized information from other sources. Since the report's publication, the sonar issue has continued to attract widespread public attention. *See, e.g.*, "Protest Raised over New Tests of Naval Sonar," National Public Radio, *All Things Considered*, July 24, 2007.

- (5) NRDC scientists have used information obtained through FOIA to publish analyses of the United States' and other nations' nuclear weapons programs. In 2004, for example, NRDC scientists incorporated information obtained through FOIA into a feature article on the United States' plans to deploy a ballistic missile system and the implications for global security. *See* Hans M. Kristensen, Matthew G. McKinzie, and Robert S. Norris, "The Protection Paradox," *Bulletin of Atomic Scientists*, Mar./Apr. 2004.
- (6) NRDC has used White House documents obtained through FOIA and from other sources to inform the public about EPA's failures to protect wildlife and workers from the pesticide atrazine in the face of industry pressure to keep atrazine on the market. *See* <http://www.nrdc.org/health/atrazine/files/atrazine10.pdf>; *see also* William Souder, "It's Not Easy Being Green: Are Weed-Killers Turning Frogs Into Hermaphrodites?" *Harper's Bazaar*, Aug. 1, 2006 (referencing documents obtained and posted online by NRDC).
- (7) NRDC has obtained, through FOIA and other sources, information on the levels of arsenic in drinking water supplies across the country. NRDC synthesized that information into a report, *Arsenic and Old Laws* (2000), printed and made available online through NRDC's website, *see* <http://www.nrdc.org/water/drinking/arsenic/aolinx.asp>, and provided analysis describing its significance and guiding interested members of the public on how to learn more about arsenic in their own drinking water supplies. *Id.*; *see also* Steve LaRue, "EPA Aims to Cut Levels of Arsenic in Well Water," *San Diego Union-Tribune*, June 5, 2000, at B1 (referencing NRDC report).
- (8) In 2000, NRDC used information obtained through FOIA to publish a report analyzing the impacts of manure pollution from large livestock feedlots on human health, fish and wildlife. *See* NRDC, *Spills & Kills*, Aug. 2000.
- (9) In 1999, NRDC obtained, through FOIA, a Defense Department document, *History of the Custody and Deployment of Nuclear Weapons: July 1945 through September 1977*. The document attracted significant press attention once it was disclosed. *See, e.g.*, Walter Pincus, "Study Says U.S. Secretly Placed Bombs; Cold War Deployments Affected Mostly Allies," *Washington Post* (Oct. 20, 1999) at A3. One of NRDC's nuclear scientists, Robert Norris, published a detailed analysis of this document explaining its significance to the public. *See* Robert S. Norris, William M. Arkin, and William Burr, "Where They Were," *Bulletin of Atomic Scientists*, Nov./Dec. 1999.
- (10) In 1996, NRDC obtained, through FOIA, test results regarding lead levels in the District of Columbia's drinking water supplies. NRDC made the test results public along with analysis explaining the significance of the results. *See* D'Vera Cohn, "Tap

Water Safeguards Still Stalled; City Failed to Tell Some Residents of Excess Lead Contamination,” *Washington Post*, Apr. 18, 1996, at J1.

- (11) In 1989, NRDC obtained, through FOIA, testimony, previously suppressed by the first Bush administration, by federal experts who opposed oil drilling off the coasts of California and Florida. *See* Larry Liebert, “Oil Testimony Reportedly Quashed; Environmentalists say Federal Experts Pressured by Bush,” *Orange County Register*, Oct. 5, 1989, at A6.
- (12) In 1988, NRDC obtained, through FOIA, a report by the U.S. Fish and Wildlife Service that declared that the government’s review of offshore oil drilling in Northern California was incomplete and overly optimistic. Reagan administration officials had tried to keep the report secret and then repudiated it upon its release. *See* Eric Lichtblau, “Federal Report Blasts Offshore Oil Studies,” *L.A. Times*, June 4, 1988, at A32.
- (13) In 1982, NRDC obtained, through a FOIA request, an EPA memorandum stating that most air pollution monitors have repeatedly underestimated levels of toxic lead in the air. NRDC used the memorandum to inform the public about the consequences of EPA’s proposal to relax restrictions on lead in gasoline. *See* Sandra Sugawara, “Lead in Air is Undermeasured, EPA Section Chief’s Memo Says,” *Washington Post*, July 11, 1982, at A6.2

As these examples demonstrate, NRDC has a proven ability to digest, synthesize, and quickly disseminate information gleaned from FOIA requests to a broad audience of interested persons. Therefore, the requested records disclosure is likely to contribute to the public’s understanding of the subject.

#### **4. *Significance of the contribution to public understanding***

The records requested shed light on a matter of considerable public interest and concern: GRAS notices for additives use in food.

Public understanding of food safety would be significantly enhanced by disclosure of the requested records concerning GRAS notices. Disclosure would help the public to more effectively evaluate food safety. Disclosure would also help the public to better understand and evaluate FDA’s actions (or inaction) on GRAS notices.

#### **B. NRDC Satisfies the Second Fee Waiver Requirement**

Disclosure in this case would also satisfy the second prerequisite of a fee waiver request because NRDC does not have any commercial interest that would be furthered by the requested disclosure. 5 U.S.C. § 552(a)(4)(A)(iii); 21 C.F.R. § 20.46(c). NRDC is a not-for-profit organization and does not act as a middleman to resell information obtained under FOIA. “Congress amended FOIA to ensure that it be ‘liberally construed in favor of waivers for noncommercial requesters.’”

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<sup>2</sup> Information NRDC obtained through FOIA requests resulted in other articles, in addition to those referenced above, *see, e.g.*, Felicity Barringer, “Science Panel Issues Report on Exposure to Pollutant,” *N.Y. Times*, Jan. 11, 2005; Katharine Q. Seelye, “Draft of Air Rule is Said to Exempt Many Old Plants,” *N.Y. Times*, Aug. 22, 2003; Don Van Natta, Jr., “E-Mail Suggests Energy Official Encouraged Lobbyist on Policy,” *N.Y. Times*, Apr. 27, 2002.

*Judicial Watch, Inc. v. Rossotti*, 326 F.3d 1309, 1312 (D.C. Cir. 2003) (internal citation omitted); *see Natural Res. Def. Council v. United States Env'tl. Prot. Agency*, 581 F. Supp. 2d 491, 498 (S.D.N.Y. 2008). NRDC wishes to serve the public by reviewing, analyzing and disclosing newsworthy and presently non-public information about GRAS notices. As noted at Part II.A, any work done by FDA on GRAS notices relates to a matter of considerable public interest and concern. Disclosure of the requested records will contribute significantly to public understanding of GRAS notices and associated threats to human health and the environment.

### C. NRDC Is a Media Requester

Even if FDA denies a public interest waiver of all costs and fees, NRDC is a representative of the news media entitled to a reduction of fees under FOIA, 5 U.S.C. § 552(a)(4)(A)(ii), and FDA's FOIA regulations, 21 C.F.R. § 20.45(a)(2). *See Elec. Privacy Info. Ctr. v. Dep't of Def.*, 241 F. Supp. 2d 5, 6, 11-15 (D.D.C. 2003) (a "non-profit public interest organization" qualifies as a representative of the news media under FOIA where it publishes books and newsletters on issues of current interest to the public); Letter from Alexander C. Morris, FOIA Officer, United States Dep't of Energy, to Joshua Berman, NRDC (Feb. 10, 2011) (granting NRDC media requester status).

NRDC is in part organized and operated to publish or transmit news to the public. As described earlier in this request, NRDC publishes a quarterly magazine, *OnEarth*, which has approximately 150,000 subscribers, is available at newsstands and bookstores, and has won numerous news media awards, including the Independent Press Award for Best Environmental Coverage and for General Excellence, a Gold Eddie Award for editorial excellence among magazines, and the Phillip D. Reed Memorial Award for Outstanding Writing on the Southern Environment. NRDC also publishes a regular newsletter for its more than one million members and online activists; issues other electronic newsletters, action alerts, public reports and analyses; and maintains free online libraries of these publications. NRDC maintains a significant additional communications presence on the internet through its staff blogging site, "Switchboard," which is updated daily and features more than 130 bloggers writing about current environmental issues, and through daily news messaging on "Twitter" and "Facebook." *See* OPEN Government Act of 2007, Pub. L. No. 110-175, § 3, 121 Stat. 2524 (2007) (codified at 5 U.S.C. § 552(a)(4)(A)(ii)) (clarifying that "as methods of news delivery evolve . . . such alternative media shall be considered to be news-media entities"). The aforementioned publications and media sources routinely include information about current events of interest to the readership and the public. To publish and transmit this news content, NRDC employs approximately thirty staff dedicated full-time to communications with the public, including accomplished journalists and editors, *see* list of select communications staff at <http://www.nrdc.org/about/staff.asp>. These staff rely on information acquired under FOIA and through other means. That NRDC is a public interest advocacy organization is inconsequential so long as "its activities qualify as those of a representative of news media," and NRDC's do. *Elec. Privacy Info. Ctr.*, 241 F. Supp. 2d at 12. Public interest organizations meeting the requirements "are regularly granted news representative status." *Serv. Women's Action Network v. Dep't of Def.*, 888 F. Supp. 2d 282, 287-88 (D. Conn. 2012) (according media requester status to the American Civil Liberties Union).<sup>3</sup>

Information obtained as a result of this request will, if appropriately newsworthy, be synthesized with information from other sources and used by NRDC to create and disseminate unique articles, reports, analyses, blogs, tweets, emails, and/or other distinct informational works through one or more of NRDC's publications or other suitable media channels. NRDC staff gather information

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<sup>3</sup> To be a representative of the news media, an organization need not *exclusively* perform news gathering functions. If that were required, major news and entertainment entities like the National Broadcasting Company (NBC) would not qualify as representatives of the news media. This country has a long history, dating back to its founding, of news organizations engaging in public advocacy.



from a variety of sources—including documents provided pursuant to FOIA requests—to write original articles and reports that are featured in its *OnEarth* magazine, newsletters, blogs, and other NRDC-operated media outlets. NRDC seeks the requested records to aid its own news-disseminating activities by obtaining, analyzing, and distributing information likely to contribute significantly to public understanding, not to resell the information to other media organizations.

### **III. Willingness to Pay Fees Under Protest**

Please provide the records requested above irrespective of the status and outcome of your evaluation of NRDC's fee category status and fee waiver request. In order to prevent delay in FDA's provision of the requested records, NRDC states that it will, if necessary and under protest, pay fees in accordance with FDA's FOIA regulations at 21 C.F.R. § 20.45 for all or a portion of the requested records. Please consult with NRDC, however, before undertaking any action that would cause the fee to exceed \$500. Such payment will not constitute any waiver of NRDC's right to seek administrative or judicial review of any denial of its fee waiver request and/or rejection of its fee category assertion.

### **IV. Conclusion**

We trust that, in responding to this request, FDA will comply with all relevant deadlines and other obligations set forth in FOIA and FDA's FOIA regulations. *See, e.g.*, 21 C.F.R. Part 20.

Please produce the records above by emailing or mailing them to me at the NRDC office address listed below. Please produce them on a rolling basis; at no point should FDA's search for—or deliberations concerning—certain records delay the production of others that FDA has already retrieved and elected to produce. In the event that FDA concludes that some of the records requested above may already be publicly available, we will be happy to discuss those conclusions. Please do not hesitate to call or email with questions.

Please do not hesitate to call or email with questions. I can be reached at 202-513-6252 and [tneltner@nrdc.org](mailto:tneltner@nrdc.org).

Thank you for your prompt attention to this request.

Sincerely,



Tom Neltner, Senior Attorney  
Natural Resources Defense Council, Inc.  
1152 15th Street NW, Suite 300  
Washington, DC 20005  
202-513-6252  
(202) 289-1060 FAX  
[tneltner@nrdc.org](mailto:tneltner@nrdc.org)

Appendix A: Generally Recognized as Safe (GRAS) Notices and Agency Actions

**APPENDIX A**  
**GENERALLY RECOGNIZED AS SAFE (GRAS) NOTICES AND AGENCY ACTIONS<sup>1</sup>**

GRN #	Title
<a href="#">1</a>	Soy isoflavone extract
<a href="#">35</a>	Hempseed oil
<a href="#">36</a>	Chromium picolinate; <i>Ginkgo biloba</i> leaf extract; and Ginseng extract
37	Whey protein isolate and dairy product solids
<a href="#">59</a>	Hydrogenated starch hydrolysate
<a href="#">66</a>	Milk thistle extract
<a href="#">150</a>	Glucosamine hydrochloride prepared from chitin obtained from <i>Aspergillus niger</i>
<a href="#">224</a>	trans-Resveratrol
<a href="#">225</a>	Catechins from green tea extract
<a href="#">257</a>	<i>gamma</i> -Amino butyric acid
<a href="#">262</a>	Sweet lupin protein
<a href="#">263</a>	Sweet lupin fiber
<a href="#">264</a>	Sweet lupin flour
<a href="#">295</a>	Aqueous extract of <i>Emblica officinalis</i>
322	Aqueous extract of <i>Emblica officinalis</i>
<a href="#">324</a>	Heat-killed <i>Lactobacillus plantarum</i>
<a href="#">340</a>	Theobromine
<a href="#">362</a>	Levocarnitine
378	Cultured [dairy sources, sugars, wheat, malt, and fruit- and vegetable-based sources] fermented by [ <i>Streptococcus thermophilus</i> , <i>Bacillus coagulans</i> , <i>Lactobacillus acidophilus</i> , <i>Lactobacillus paracasei</i> subsp. <i>paracasei</i> , <i>Lactobacillus plantarum</i> , <i>Lactobacillus sakei</i> , <i>Lactobacillus bulgaricus</i> and <i>Propionibacterium freudenreichii</i> subsp. <i>shermanii</i> or mixtures of these strains]
<a href="#">444</a>	Milk protein concentrate and milk protein isolate

<sup>1</sup> Posted at <http://www.accessdata.fda.gov/scripts/fcn/fcnNavigation.cfm?rpt=grasListing>.





DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration  
Silver Spring MD 20993

tneltner@nrdc.org

In Reply Refer To: FOI 2013-8042

Dear Requester:

This is in partial response to your October 11, 2013, request to the Food and Drug Administration (FDA) pursuant to the Freedom of Information Act for records regarding:

GRN 1,35,36 ETC

A search of the Office of the Commissioner, Office of the Executive Secretariat files did not reveal any responsive records to your request.

If you wish to appeal from this determination, please submit your appeal within 30 days to Director, News Division, 7700 Wisconsin Avenue, Suite 920, Bethesda, MD 20857 (by U.S. Post), or 7700 Wisconsin Avenue, Suite 920, Bethesda, MD 20814 (by private courier, such as UPS or FedEx). Please mark your envelope FDA FOIA Appeal and please include your control number.

Sincerely,

A handwritten signature in blue ink, reading "Martina H. Varnado", is written over a horizontal line. To the left of the signature, there is a small "x" mark.

Martina H. Varnado  
Director  
Office of the Commissioner  
Office of the Executive Secretariat



DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration  
College Park, MD 20740

January 16, 2014

Tom Neltner  
Natural Resources Defense Council  
1152 15th Street, N.W. Suite 300  
Washington, DC 20005

Re: FOI Request No. 2013-8042

Dear Mr. Neltner:

This is in response to your request of October 21, 2013, requesting records related to GRAS Notices 1, 35, 36, 37, 59, 66, 150, 224, 225, 257, 262, 263, 264, 295, 322, 324, 340, 362, 378, and 444. In this response, we have provided responsive records for 19 of the 20 GRAS Notices you requested. We are compiling responsive records for GRAS Notice 1 and will provide these at a later date. Per your request, we do not include copies of the notices and agency letters posted at <http://www.accessdata.fda.gov/scripts/cfn/fcnNavigation.cfm?rpt=grasListing> in our response. We do, however, include in our response documents available in the Federal Register or Docket FDA-1997-N-0020 to ensure completeness of the responsive records. Your request was forwarded to the Office of Food Additive Safety in the Center for Food Safety and Applied Nutrition.

X Enclosed is a partial response of the records you requested (with the exception of responsive records for GRAS Notice 1).

X Certain material has been deleted from the records furnished to you because a preliminary review of the records indicated that the deleted information is not required to be publicly disclosed and that disclosure is not appropriate. FDA has taken this approach to facilitate the process of responding to you. If you dispute FDA's preliminary determination with respect to these records and would like FDA to reconsider any particular deletion, please let us know in writing at the following address: Food and Drug Administration, Division of Freedom of Information, HFI-35, 5600 Fishers Lane, Rockville, MD 20857 within 30 days from the date of this letter. If we do not receive a response in that time period, we will consider the matter closed with respect to these records. If you do request further consideration and if the agency then formally denies your request for any or all of the previously-withheld information, you will have the right to appeal that decision. Any letter of denial will explain how to make this appeal.

The following charges for this request to date may be included in a monthly invoice:

Reproduction \$ 0.00 Search \$0.00 Review \$0.00 Other \$0.00 (CD) Total \$0.00

THE ABOVE TOTAL MAY NOT REFLECT THE FINAL CHARGES FOR THIS REQUEST. **PLEASE DO NOT SEND PAYMENT UNTIL YOU RECEIVE AN INVOICE FOR THE TOTAL MONTHLY FEE.**

Sincerely Yours,

Sharon R. Dodson

Sharon R. Dodson  
Program Analyst  
Office of Food Additive Safety  
Center for Food Safety  
and Applied Nutrition

Enclosure





DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration  
College Park, MD 20740

March 19, 2014

Tom Neltner  
Natural Resources Defense Council  
1152 15th Street, N.W. Suite 300  
Washington, DC 20005

Re: FOI Request No. 2013-8042

Dear Mr. Neltner:

This completes our response to your request of October 21, 2013, requesting records related to GRAS Notices 1, 35, 36, 37, 59, 66, 150, 224, 225, 257, 262, 263, 264, 295, 322, 324, 340, 362, 378, and 444. Per your request, we do not include copies of the notice and agency letter posted at <http://www.accessdata.fda.gov/scripts/fcn/fcnNavigation.cfm?rpt=grasListing> in our response. We do, however, include in our response documents available in Docket FDA-1997-N-0020 to ensure completeness of the responsive records. Your request was forwarded to the Office of Food Additive Safety in the Center for Food Safety and Applied Nutrition.

In this response, we provide Part 1 of 2 of the responsive records for GRAS Notice 1, thus completing our response for this notice.

☒ Enclosed is Part 1-GRAS Notice 1 of the records you requested.

☒ Certain material has been deleted from the records furnished to you because a preliminary review of the records indicated that the deleted information is not required to be publicly disclosed and that disclosure is not appropriate. FDA has taken this approach to facilitate the process of responding to you. If you dispute FDA's preliminary determination with respect to these records and would like FDA to reconsider any particular deletion, please let us know in writing at the following address: Food and Drug Administration, Division of Freedom of Information, HFI-35, 5600 Fishers Lane, Rockville, MD 20857 within 30 days from the date of this letter. If we do not receive a response in that time period, we will consider the matter closed with respect to these records. If you do request further consideration and if the agency then formally denies your request for any or all of the previously-withheld information, you will have the right to appeal that decision. Any letter of denial will explain how to make this appeal.

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THE ABOVE TOTAL MAY NOT REFLECT THE FINAL CHARGES FOR THIS REQUEST. **PLEASE DO NOT SEND PAYMENT UNTIL YOU RECEIVE AN INVOICE FOR THE TOTAL MONTHLY FEE.**

Sincerely Yours,

Sharon R. Dodson

-S

Sharon R. Dodson  
Program Analyst  
Office of Food Additive Safety  
Center for Food Safety  
and Applied Nutrition

Signature required by Standard & Daniels  
DHEC-001, 0011, 0012, 0013, 0014, 0015, 0016, 0017, 0018, 0019, 0020, 0021, 0022, 0023, 0024, 0025, 0026, 0027, 0028, 0029, 0030, 0031, 0032, 0033, 0034, 0035, 0036, 0037, 0038, 0039, 0040, 0041, 0042, 0043, 0044, 0045, 0046, 0047, 0048, 0049, 0050, 0051, 0052, 0053, 0054, 0055, 0056, 0057, 0058, 0059, 0060, 0061, 0062, 0063, 0064, 0065, 0066, 0067, 0068, 0069, 0070, 0071, 0072, 0073, 0074, 0075, 0076, 0077, 0078, 0079, 0080, 0081, 0082, 0083, 0084, 0085, 0086, 0087, 0088, 0089, 0090, 0091, 0092, 0093, 0094, 0095, 0096, 0097, 0098, 0099, 0100, 0101, 0102, 0103, 0104, 0105, 0106, 0107, 0108, 0109, 0110, 0111, 0112, 0113, 0114, 0115, 0116, 0117, 0118, 0119, 0120, 0121, 0122, 0123, 0124, 0125, 0126, 0127, 0128, 0129, 0130, 0131, 0132, 0133, 0134, 0135, 0136, 0137, 0138, 0139, 0140, 0141, 0142, 0143, 0144, 0145, 0146, 0147, 0148, 0149, 0150, 0151, 0152, 0153, 0154, 0155, 0156, 0157, 0158, 0159, 0160, 0161, 0162, 0163, 0164, 0165, 0166, 0167, 0168, 0169, 0170, 0171, 0172, 0173, 0174, 0175, 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1836, 18



DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration  
College Park, MD 20740

March 19, 2014

Tom Neltner  
Natural Resources Defense Council  
1152 15th Street, N.W. Suite 300  
Washington, DC 20005

Re: FOI Request No. 2013-8042

Dear Mr. Neltner:

This completes our response to your request of October 21, 2013, requesting records related to GRAS Notices 1, 35, 36, 37, 59, 66, 150, 224, 225, 257, 262, 263, 264, 295, 322, 324, 340, 362, 378, and 444. Per your request, we do not include copies of the notice and agency letter posted at <http://www.accessdata.fda.gov/scripts/fcn/fcnNavigation.cfm?rpt=grasListing> in our response. We do, however, include in our response documents available in Docket FDA-1997-N-0020 to ensure completeness of the responsive records. Your request was forwarded to the Office of Food Additive Safety in the Center for Food Safety and Applied Nutrition.

In this response, we provide Part 2 of 2 of the responsive records for GRAS Notice 1, thus completing our response for this notice.

☒ Enclosed is Part 1-GRAS Notice 1 of the records you requested.

☒ Certain material has been deleted from the records furnished to you because a preliminary review of the records indicated that the deleted information is not required to be publicly disclosed and that disclosure is not appropriate. FDA has taken this approach to facilitate the process of responding to you. If you dispute FDA's preliminary determination with respect to these records and would like FDA to reconsider any particular deletion, please let us know in writing at the following address: Food and Drug Administration, Division of Freedom of Information, HFI-35, 5600 Fishers Lane, Rockville, MD 20857 within 30 days from the date of this letter. If we do not receive a response in that time period, we will consider the matter closed with respect to these records. If you do request further consideration and if the agency then formally denies your request for any or all of the previously-withheld information, you will have the right to appeal that decision. Any letter of denial will explain how to make this appeal.

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Sincerely Yours,

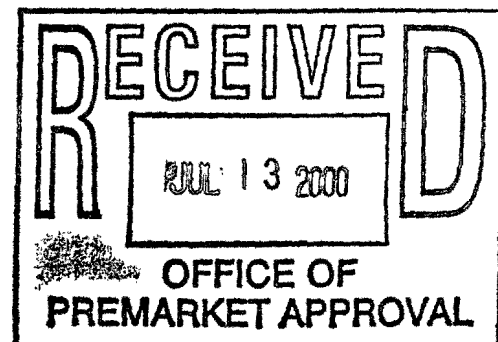
Sharon R.  
Dodson -S  
Sharon R. Dodson  
Program Analyst  
Office of Food Additive Safety  
Center for Food Safety  
and Applied Nutrition

Enclosure

PR



**SELF GRAS NOTIFICATION FOR  
HYDROGENATED STARCH HYDROLYSATE**



000001

**NOTIFICATION OF  
SELF GRAS DETERMINATION**

Name of Petitioner : Grain Processing Corporation  
and  
SPI Polyols Inc.

Address : All communications on this matter are to be sent to :  
Ms. Rani Thomas  
Director of Quality and Regulatory Affairs  
Grain Processing Corporation  
1600 Oregon Street  
Muscatine, IA 52761

Name of Generally Recognized as safe  
Substance(GRAS) Hydrogenated Starch Hydrolysate

Dated : June 30, 2000

**000002**

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**000003**



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**000005**



## GRAIN PROCESSING CORPORATION

1600 Oregon St., Muscatine, Iowa 52761-1494 USA ■ Phone 319-264-4265 ■ FAX 319-264-4289

June 30, 2000

Office of Premarket Approval (HFS-200)  
Center for Food Safety and Applied Nutrition  
Food and Drug Administration  
200 C St. S.W.  
Washington, DC 20204

Re: A Notice of a Self- GRAS determination for hydrogenated malto-oligosaccharide (Hydrogenated Starch Hydrolysate)

Dear Sir:

In accordance with the notification procedure (proposed 21 CFR 170.36), Grain Processing Corporation (GPC) in Muscatine, Iowa, and SPI Polyols (SPI) in New Castle, Delaware, submit this notification for Self-GRAS determination of hydrogenated malto-oligosaccharides which fits within the definition of hydrogenated starch hydrolysate. As in the proposed regulation by FDA, GPC/SPI are submitting the following information to establish the use of this substance is GRAS.

### Hydrogenated Starch Hydrolysate

- a) HSH is a concentrated, aqueous solution of sorbitol ( $C_6 H_{14} O_6$ ), maltitol ( $C_{12} H_{24} O_{11}$ ), maltitriol ( $C_{18} H_{34} O_{16}$ ) and hydrogenated polysaccharides. It is produced by the transition metal catalytic hydrogenation of glucose syrup or polymers of glucose.
- b) The GPC/SPI ingredients meet the following specifications:  
total solids not less than 50% for the liquid product and not less than 90% for the dry product; sorbitol not more than 10%, maltitol not more than 10%, hydrogenated tri to hexasaccharides between 5 and 35 %, hydrogenated saccharides higher than hexa greater than 50%; arsenic (As) not more than 10 parts per million; chloride not more than 50 parts per million; heavy metals (such as lead) not more than 10 parts per million; reducing sugars not more than 1.0%, residue on ignition; not more than 0.1%; sulfate not more than 100 parts per million; total sugars (after hydrolysis) not more than 97% on the dry basis.
- c) The ingredient is used as a flavoring agent and adjuvant as defined in CFR 170.3 (o) of this chapter; formulation aid as defined in CFR 170.3 (o)(14) of this chapter; humectant as defined in CFR 170.3 (o)(21) of this chapter, processing aid as defined in CFR 170.3

000006

(o)(24) of this chapter, stabilizer and thickener as defined in CFR 170.3 (o)(28) of this chapter; surface-finishing agent as defined in CFR 170.3 (o) (30) of this chapter; and texturizer as defined in CFR 170.3 (o) (32) of this chapter.

- d) The ingredient is used in food at levels not to exceed good manufacturing practices. Current practices in the use of HSH result in maximum levels of:

Food Use:	Liquid HSH	Dry HSH
Hard Candy	99%	60%
Soft Candy	90%	50%
Chewing Gum	25%	45%
Bakery	35%	25%
Ice cream	20%	15%

Grain Processing Corporation and SPI Polyols will market HSH under several trade names. Grain Processing Corporation is headquartered and has manufacturing facilities in Muscatine, Iowa and Washington, Indiana. SPI Polyols, Inc. is headquartered and has manufacturing facilities in New Castle, Delaware.

Detailed information on the chemistry, composition and manufacture of this hydrogenated starch hydrolysate (HSH) is presented in the text. This hydrogenated starch hydrolysate is a mixture of polysaccharides of varying chain length where 0 - 10% is sorbitol, 0 - 10% is maltitol, and greater than 30% is hydrogenated saccharides with 8 or more units.

#### **Common use in Foods**

The GRAS determination is based on the common use of HSH in foods. This substance has been used in foods for long periods of time. It has been marketed extensively by Roquette and Lonza for years. We have reviewed the scientific data submitted by Roquette Freres in their petition for hydrogenated glucose syrup. Our process is essentially equivalent to the process of Lonza and Roquette Freres. The HSH products produced by Roquette Freres and Lonza have been used in food for more than ten years. In support of the GRAS filing of this new hydrogenated starch hydrolysate, Grain Processing Corporation and SPI Polyols will site data submitted by Roquette in its Lycasin® 80/55 petition (84G-003) regarding numerous studies relating to the safety of the ingredient, including reports on

- Digestion, absorption, distribution and excretion
- Subchronic toxicity
- Genetic toxicity
- Reproduction
- Biological tolerance

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- Human exposure
- Acute oral LD<sub>50</sub>
- Laxation effect

An acute oral LD<sub>50</sub> study of this new HSH is included in Appendix E.

It is our belief that the definition of an HSH fits the description of our product, hydrogenated malto-oligosaccharides as GPC/SPI products are derived from cornstarch, and are hydrolyzed to a starch hydrolysate and hydrogenated using the same technology and methods used to produce other HSH products. GPC/SPI products have less DP1,2, and 3 in comparison to other available HSH products in that they are less hydrolysed than existing Hydrogenated Starch Hydrolysates. See attached report comparing the Roquette, Lonza and GPC processes (Attachment v on page 16).

Citing data contained in the Lycasin® and Hystar® petitions and chemistry data supplied in this petition, it can be predicted that the new HSH products produced by GPC/SPI will be digested similarly and broken down in the digestive tract into its two GRAS congeners glucose and sorbitol. The GPC/SPI product should have a reduced laxative effect due to the lesser amount of sorbitol present in the HSH product. Since the GPC/SPI HSH products differ from existing HSH products only in the carbohydrate profile, it is our technical opinion that the GPC/SPI products do not pose any toxicological and health concerns.

Dr. Robert Linhardt, Professor, Departments of Chemistry, Pharmacy and Chemical Engineering of the University of Iowa has reviewed the GRAS information submitted by Lonza, Roquette and the GRAS information put together by Grain Processing Corporation and SPI Polyols. According to Dr. Linhardt, the GPC/SPI product(s) is sufficiently similar in chemical composition to the Lonza product to make it behave identically with respect to the biological evaluation required for GRAS approval. On the basis of the data provided this appears to be the case. In particular the GPC product is clearly very similar to the Lonza product except for its somewhat higher theoretical molecular weight, permitting the conclusion that these products are biologically equivalent. A report from Dr. Linhardt is attached to this document (Attachment vi on page 19).

Please call me if you have any questions.

Thanking you,  
Yours sincerely



Rani M. Thomas  
Director of Quality and Regulatory Affairs

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**i. Description of the substance**

**000011**

i. Description of the GPC/SPI Substance

(a) Common or Usual Name

The principle common name of the product is hydrogenated starch hydrolysate (HSH). Other names that have also been used include hydrogenated glucose syrup, hydrogenated glucose solids, reduced malto-oligosaccharide, stabilised carbohydrate polymer and polyalditol.

(b) Chemical Name

A mixture of sorbitol (1,2,3,4,5,6-hexol hexane), maltitol (4-O- $\alpha$ -D-glucopyranosyl-D-glucitol), and hydrogenated polysaccharides containing greater than 3 D-glucopyranosyl units joined by  $\alpha$ -1-4 linkages and terminated with a D-glucityl unit.

(c) Chemical Abstracts Service (CAS) Registry Number

The CAS number of hydrogenated starch hydrolysate is 68425-17-2.

(d) Empirical Formulae

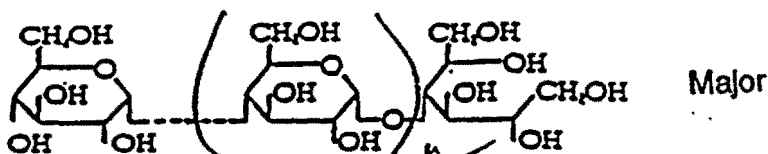
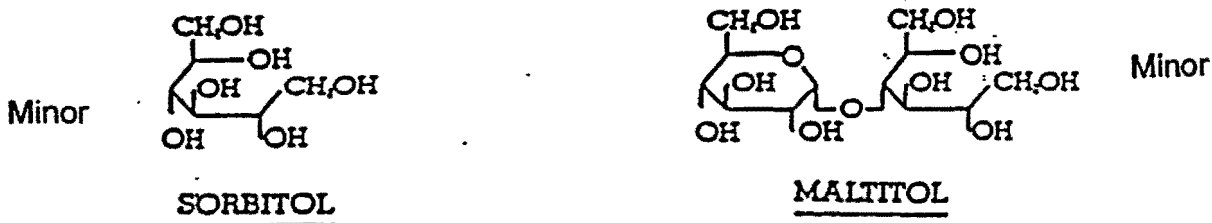
The empirical formulae of the components of hydrogenated starch hydrolysate are:

Sorbitol:	$C_6H_{14}O_6$
Maltitol:	$C_{12}H_{24}O_{11}$
Hydrogenated Polysaccharides:	$C_{12}H_{24}O_{11}$ plus $C_6H_{10}O_5$ For each additional glucose moiety in the chain.

The theoretical molecular weight of the hydrogenated starch hydrolysates ranges from approximately 1000-3600.

(e) Structural Formulae

The structural formulae of the components are:



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(f) Specifications for Food-Grade Material

The specifications for food-grade hydrogenated starch hydrolysate are as follows:

Total Solids	Not less than 90% for dry product Not less than 50% for liquid product
Sorbitol	Not more than 10% (dry product basis)
Maltitol	<10%
Hydrogenated Tri- to hexasaccharides	Between 5 and 35% (dry product basis)
Hydrogenated saccharides higher than hexa	greater than 50% (dry product basis)
Arsenic (as As)	Not more than 10 ppm
Chloride	Not more than 50 ppm
Heavy metals (as Pb)	Not more than 10 ppm
Reducing sugars	Not more than 1%
Residue on ignition	Not more than 0.1%
Sulfate	Not more than 100 ppm
Total sugars (after Hydrolysis)	Not more than 97% (dry product basis)

The methods to be used in determining compliance with the specifications are provided in Appendix A (Part 1), and the results of the analysis of representative lots of hydrogenated starch hydrolysate are provided in Appendix A (Part 2).

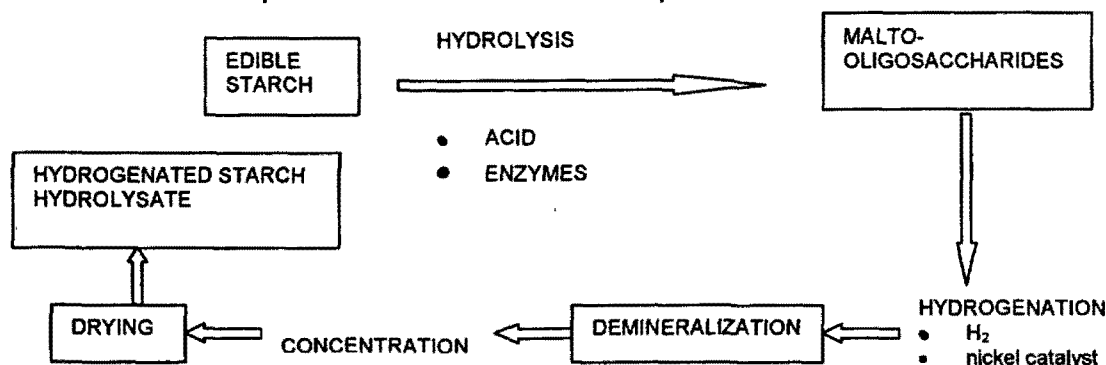
(g) Quantitative Composition

Hydrogenated starch hydrolysate consists of approximately 5% water and approximately 95% total solids for dry product, of which not more than 10% is sorbitol, not more than 10% is maltitol, and the remainder is hydrogenated polysaccharides. For liquid product the solids is >50%.

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(h) Manufacturing Process

The manufacturing process used to produce hydrogenated starch hydrolysates are the same as that used to produce sorbitol, as shown below and as presented in greater detail in Appendix A (Part 3). No new or novel procedures are used in this process.



More specifically, food grade edible starch is enzymatically hydrolyzed by using alpha-amylase and acid to reach 4-35 dextrose equivalent liquid malto-oligosaccharides.

The liquid malto-oligosaccharides are hydrogenated by using nickel catalyst. After hydrogenation, the liquid hydrogenated starch hydrolysate is filtered, demineralized on ion-exchange resins. The material can be concentrated to 50 percent or greater dry substance syrup or dried to a powder of approximately 5% moisture.

The products produced by this method consist of hydrogenated starch hydrolysates corresponding to the range of saccharides identified in the original liquid malto-oligosaccharides, since the conversion of the sugars by hydrogenation of their end reducing units is virtually complete. During the hydrogenation process only the dextrose and reducing end units in the oligo- and higher saccharides are converted into sorbitol units and glucityl units as shown diagrammatically in Appendix A (Part 3).

In addition to the materials and chemicals mentioned above, lime, sodium hydroxide, sulfuric acid, active carbon, soda ash and filtration earth are also used in the manufacturing process, all of which are commonly used in glucose-dextrose and sorbitol manufacture.

The product obtained by the process described above is a colorless, odorless, bland tasting liquid or powder. A table on typical properties is included in Appendix A (part 2).

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II. Use of Hydrogenated  
Starch Hydrolysate

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ii. **Use of Hydrogenated Starch Hydrolysate**

**000016**

ii. Use of Hydrogenated Starch Hydrolysate

(a) Date when Use Began

Information available to us indicates that Roquette began marketing Lycasin® 80/55 in the United States in 1977, since which time it has been used in hard candies on the basis of self-determination of its GRAS status by candy manufacturers. In a similar manner, Lonza submitted petition in 1985 for their HSH products. GPC and SPI began laboratory testing Hydrogenated Starch Hydrolysate in the United States in 1997. The major components of Hydrogenated Starch Hydrolysate, i.e., maltitol and hydrogenated polysaccharides, however occur at relatively low levels in the GRAS substance sorbitol, which was first used in food in the United States more than 50 years ago. A chromatogram illustrating the carbohydrate components of Hydrogenated Starch Hydrolysate is presented in Appendix A Part 5.

(b) Information on Past Uses in Food

Roquette's petition indicates that Roquette's Lycasin® products have been approved for use in food in Europe since 1963, as indicated below.

Country	Year of Approval
Sweden	1963 (reaffirmed in 1975)
Switzerland	1968
Norway	1975
Finland	1975 (reaffirmed in 1980)
Denmark	1976

(c) Foods in Which Used, Levels of Use, and For What Purpose

Current practices in the use of HSH result in maximum levels of:

Food Use:	Liquid HSH	Dry HSH
Hard Candy	99%	60%
Soft Candy	90%	50%
Chewing Gum	25%	45%
Bakery	35%	25%
Ice cream	20%	15%

Hydrogenated Starch Hydrolysate has a unique combination of desirable properties, including high viscosity, hydroscopicity, binding capacity, anticrystallizing capacity, low reactivity and is stable under all normal-manufacturing conditions.

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iii. **Methods for Detecting Hydrogenated Starch Hydrolysate**

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iii. Methods for Detecting Hydrogenated Starch Hydrolysate

The qualitative analysis of Hydrogenated Starch Hydrolysate in the foods to which it has been added may be accomplished by extraction of the sorbitol and maltitol moieties with appropriate solvents, followed by gas chromatography of the extracts. Similarly, the quantity of hydrogenated starch hydrolysate occurring in food may be estimated by determining the amount of maltitol recovered and applying an appropriate factor. Complete details, including information on the sensitivity and reproducibility of the method, are provided in Appendix D.

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**iv. INFORMATION TO ESTABLISH GENERAL RECOGNITION OF HSH IN  
FOOD**

**000022**

## SAFETY

The following is in regard to the Safety of Grain Processing Corporation and SPI Polyols hydrogenated starch hydrolysates (HSH) also commonly referred to as hydrogenated glucose syrups, hydrogenated dextrans, reduced dextrans, reduced malto-oligosaccharides, stabilised carbohydrate and polyalditol. Per capita consumption of HSH is referenced in Appendix C.

HSH produced by Grain Processing Corporation and SPI Polyols as described in Section I - Description of the substance, intended as direct food additives, are produced by hydrogenation of GRAS malto-oligosaccharides. The resultant HSH products are completely non-toxic. The acute oral toxicity is reported at >10g/kg. See Appendix E, Exhibits S1.2 for reports of the acute oral toxicity for SPI/GPC hydrogenated starch hydrolysate.

In order to further substantiate the safe use of Hydrogenated starch hydrolysate, reference is made to Lonza Corporation GRAS affirmation Petition Submission (1985); and reference is made to Roquette Corporation GRAS affirmation Petition Submission #84G-0003. See Appendix E, Exhibit S1.0 and Exhibit S2.

SPI Polyols and Grain Processing Corporation believe that these data can be used for the safety assessment of the new hydrogenated starch hydrolysate, since the composition of GPC/SPI HSH product is significantly similar to Lonza's Hystar® and Roquette Corporation's Lycasin® products. The chronic toxicity and metabolic pathways of the new hydrogenated starch hydrolysate will be the same as those elucidated in the Lonza and Roquette petitions, thus leading to the metabolites sorbitol and glucose for which safety has been established.

Further, the new HSH contain Sorbitol, the DP – 1 hydrogenate in varying degrees but less than the Hystar® and Lycasin® products. See Table on Page 18 on carbohydrate profiles.

## Laxative Potential

Lonza in their GRAS Petition reported laxation effects of a variety of Hystar® products as shown in Appendix E, Exhibits 3.0 and 3.1. The Hystar® composition with the highest amount of DP – 1 hydrogenate (sorbitol) produced the most pronounced laxative response. Conversely, those Hystars® with larger amounts of DP – 4+ showed significantly reduced degrees of laxation. Based on these results an acceptable range of consumption of approximately 50g per day for Hystar® 7000 to 100g per day for Hystar® 6075 was established.

Based upon comparisons of carbohydrate profiles as shown on page 18, it is our position that the acceptable range of consumption will be at least 100g/day as established by Hystar® 6075, the product with the most similar carbohydrate profile.

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**HYDROGENATED STARCH HYDROLYSATE**  
**USE IN FOOD AND**  
**APPLICATION FORMULAS**

**000024**

## HYDROGENATED STARCH HYDROLYSATE USE IN FOODS

### Introduction:

This section describes the function of the new GPC/SPI HSH products as they are used in foods.

### Functionality / Applications

The new HSH products from GPC/SPI are unique from other HSH products currently in the market in the amount of the longer polysaccharide chains contained in the product. This characteristic will provide increased thickening and ease of drying over other HSH products. This functionality can expand the use of HSH for texture modification, crystallization inhibition and building solids to aid in drying. In addition, the lower amounts of the smaller molecular weight sugar alcohols in the GPC/SPI product contributes to a lower sweetness profile that is more compatible with savory flavor systems.

Similar to other HSH products currently in the market, the GPC/SPI HSH products are more stable to chemical and thermal reactions than many sugars, maltodextrin, or other carbohydrate products, due to the hydrogenation of the reducing end of the polysaccharide chains. Without the reducing group, the polysaccharide chains do not participate in Maillard Browning, nor do they caramelize until heated sufficiently to break the chains and expose reducing end groups. HSH is also generally less fermentable by microorganisms making them particularly useful in chewing gums and candies.

Applications where the GPC/SPI HSH products appear particularly useful include:

#### Food Uses

Hard Candy

Soft Candy

Chewing Gum

Chocolate Compound Coatings

Bakery Products

Fillings and Icings

Syrups

Sauces

Ice Cream

Beverage Mixes

The sample formulas listed in Appendix B are examples of food applications for the GPC/SPI HSH. These applications utilize the HSH for thickening and crystal modification.

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**v. Comparison of Roquette, Lonza and GPC/SPI processes**

**000027**

To: R. Antrim, R. Thomas

Project: 4125

From: Frank Barresi

Jan. 27, 2000

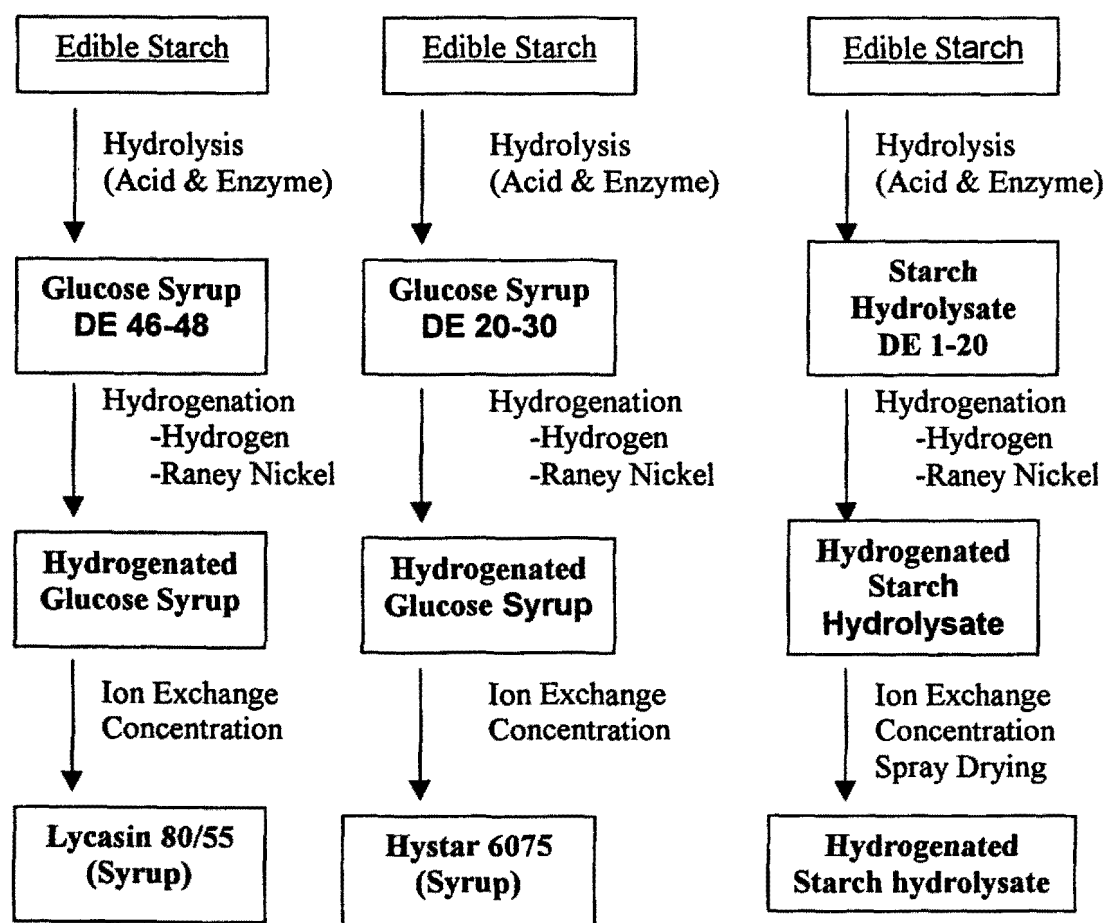
Subject: Roquette, Lonza, GPC/SPI Hydrogenated Starch Hydrolysates:

The following is a general description of the three processes used to produce hydrogenated starch hydrolysates. The Roquette process is obtained from the GRAS petition for "Hydrogenated Glucose Syrup" (Lycasin 80/55). The Lonza process is obtained from the GRAS petition for "Hydrogenated Starch Hydrolysate" (Hystar 6075). The GPC/SPI process is described in the patent application "Reduced Malto-Oligosaccharides" and has been outlined below for direct comparison to the Roquette and Lonza processes.

**Roquette – Lycasin 80/55**

**Lonza – Hystar 6075**

**GPC/SPI – Hydrogenated  
Malto oligosaccharides**



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As can be seen from the flow diagrams, the three processes follow identical pathways and are different only in the degree of starch hydrolysis. This is further outlined in the following carbohydrate profiles:

Degree of Polymerization	Roquette – Lycasin 80/55*	Lonza – Hystar 6075**	GPC/SPI Hydrogenated Starch Hydrolysate HSH A***
DP 1	8%	12-15%	0.3-3.3
DP 2	50-55	6-9	4.2-8.2
DP 3	---	8-11	6.3-10.3
DP 4	---	---	4.4-8.4
DP > 4	---	69 max.	---
DP 5	---	---	3.9-7.9
DP 6	---	---	9.4-13.4
DP 3-6	20-25	---	--
DP > 6	15-20	---	--
DP 7	---	---	7.5-11.5
DP 8	---	---	1.9-5.9
DP > 8	---	---	42.6-50.6

\* p. 10 of the Roquette GRAS petition

\*\* p. 000020 of the Lonza GRAS petition

\*\*\* p. 9 of the GPC PCT WO 99/36442– “Reduced Malto-Oligosaccharides”

cc. Project 4125 file.

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**vi. Review of properties of HSH by Dr. Linhardt, Professor of the University  
of Iowa**

**000031**

**FINAL REPORT****Review of Properties of Hydrogenated Starch Hydrolyzate (HSH) prepared by GPC,  
Roquette Freres (Lycasin) and Lonza (Hystar)**

Prepared for  
Grain Processing Corporation (GPC)  
Prepared by  
Dr. Robert J. Linhardt  
Professor, Departments of Chemistry,  
Pharmacy and Chemical Engineering

Date Submitted

February 8, 2000

**000032**

## Background

Dr. Linhardt was approached by GPC Inc. of Muscatine, Iowa to act as a consultant in the evaluation of their HSH product for its equivalency to the HSH products of Roquette Freres and Lonza. The purpose of this independent evaluation was to support a GRAS affirmation petition to the Food and Drug Administration (FDA). GPC gave oral presentations of the process used for the production of their HSH product and provided the following written documentation.

1. PCT/US99/01098 Titled: "Reduced Malto-Oigosacchaarides", published July 22, 1999 (WO99/36442), issued to GPC.
2. US Patent No. 5,493,014, February 20, 1996, Titled: "Hypocariogenic hydrogenated starch hydrolysate process for preparing it and the application of this hydrolysate issued to Roquette Freres.
3. A 1994 publication on HSH by the Caloric Control Council.
4. A letter dated April 5, 1995 from Dr. Saltsman of the FDA confirming the energy value of HSH.
5. An internal GPC document describing their HSH product, its specifications, manufacturing process, the methods used in its analysis and potential application.
6. A brochure produced by GPC describing their Maltrin product line of maltodextrins and corn syrup solids for pharmaceuticals. These maltodextrins are GRAS and represent the immediate precursor of the GPC HSH product. BPC Analytical method for determination of DP.
7. Towards a Rational Design of Commercial Maltodextrins: A Mechanistic Approach by Leon M. Marchal, 1999.
8. Viscosity data on M180 and Reduced Maltodextrins Solutions from GPC Comparison of Roquette/Lonza/GPC Processes prepared by F. Barresi of GPC 1/27/2000.
9. The GRAS Petition 5 GO304 filed by Lonza Inc. for their HSH product Hystar 6075 provided by the FDA through the Freedom of Information Act.
10. The GRAS Petition filed by Roquette Corporation for their HSH product Lycas in 80/55 provided by SPI Polyols.

## Outline of Evaluation Process

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After having read all of the material provided, three issues will be addressed: 1. Are the starting materials used in the Roquette Freres, Lonza and GPC processes the same?; 2. Will the

processes used in the preparation of these three products lead to a chemically equivalent HSH?; and 3. Are these HSH products chemically equivalent and would the chemical equivalence of these three HSH products, as demonstrated by the analytical methods applied, lead to bioequivalent products?

### **1. Equivalence of starting materials and immediate precursors of hydrogenation**

All three products are derived from edible starch which is hydrolyzed with acid and enzymes to obtain precursor for hydrogenation.

Lycasin, the Roquette Freres product, is prepared from the hydrogenation of glucose syrup. The Roquette GRAS petition shows a DE of 46-48 for the glucose syrup starting material.

Hystar 6075, the Lonza product, is prepared from the hydrogenation of glucose syrup with a dextrose equivalent (DE) 26 (DE 20-30), a viscosity of 200 P (66% solids) at 100°F (38°C) containing 5% degree of polymerization (DP)1, 14% DP2, 14% DP3 and 67% > DP4. This material corresponds to commercial glucose corn syrup prepared by acid and enzymatic hydrolysis of starch.

GPC product is prepared from the hydrogenation of starch hydrolysate. GPC starch hydrolysates have average DE values ranging from 4-7 to 16.5-19.5 (M040 to M180). The distribution of components range from 0.3% to 1.6% DP1, 0.9%-5.8% DP2, 1.4%-7.8% DP3 and 96%-80% > DP4 (M040 to M180) theoretical number average molecular weight of 3600-1000 is given for M040 and M180, respectively. A DP average for these products range from 22.1 (M040) to 6.2 (M180). The viscosity M180 (70% solids) is 80-90 P at 35°C.

Comparison of the properties of all three hydrogenation precursors clearly demonstrate that while none are chemically identical, the GPC hydrogenation precursor is most similar to the Lonza hydrogenation precursor based on DP distributions. Moreover, the Lonza hydrogenation precursor is very dissimilar to the Roquette hydrogenation precursor based on this same parameter. The differences among the three precursors is primarily associated with differences in average molecular weight suggested by the different DE values reported.

In summary, there is little (if any) difference in the structure of the components present in each of the three hydrogenation precursors. There are differences in the distribution of components, particularly between the Roquette and the Lonza hydrogenation precursors.

### **2. Equivalence of Reduction Process**

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The Roquette Freres process utilizes catalytic (Raney Nickel) hydrogenation at 130°C and 725 psi followed by filtration to remove catalyst and decolorization with activated carbon, demineralization by cationic and anionic exchange and concentration to 70-85%. No information is provided on the reaction pH and reaction time.

The Lonza process utilizes catalytic (Raney nickel) hydrogenation. No information on reaction temperature, pressure, pH or time are provided. This reaction is followed by filtration, anionic, cationic exchange and mixed bed ion exchanges, concentration, safety filtration and filling.

The GPC process utilizes catalytic (Raney nickel GD 3110) hydrogenation. The reaction product is filtered to remove catalyst, demineralized using strong cation ( $H^+$ ) and weak anion ( $OH$ ) exchange resins and following an optional activated carbon decolorization step the product is concentrated for filling.

The specific details of the GPC process are available from the documentation provided while the two other commercial processes are described in much more general terms. The GPC process is close to (or within the specifications of) the other processes suggesting an equivalency of all three processes. The advantages listed in the GPC process suggest it may represent somewhat milder hydrogenation conditions accounting for the claimed improvements of the final GPC HSH product.

### 3. Equivalence of the HSH Products

The GPC HSH product is compared to the Lonza and the Roquette Freres HSH products in Table 1. The data presented is less complete for the Lonza and Roquette Freres products than for the GPC product. The DE of the GPC product, 0.3-0.98 is slightly higher than those for the other two products < 0.2. The DP1 component for the Roquette and Lonza products are comparable while the GPC product has a slightly lower content of DP1 component. The DP2 component of the GPC product is comparable to the Lonza product but both are much lower than the DP2 component of the Roquette Freres product. The DP3 and DP>4 components for the Lonza and GPC products are comparable. The DP3-6 components of the Roquette and GPC products are similar but these products differ greatly in the DP>6 components.

In summary the GPC product most closely resembles the Lonza product, having a DP distribution that is nearly identical to this product within the accuracy of the methods used. While the GPC and Lonza products are similar to each other, they are both dissimilar in DP distribution to the Roquette Freres product. The theoretical molecular weight of the GPC product is considerably higher than that presented for either the Lonza and Roquette products

All three HSH products differ in the distribution of the components present. The structures of the identified components, however, are the same. The hydrogenation conditions used by GPC appear to be milder (The precise hydrogenation conditions for the Lonza and Roquette products are not defined in their GRAS petitions.) resulting in less breakdown of the hydrogenation precursor leading to a higher theoretical molecular weight for the GPC product. The milder hydrogenation conditions used in the GPC process would undoubtedly result in less side-products, affording a more pure GPC product.

Roquette and Lonza present considerable biological data on their HSH products aimed at establishing safety and functionality. These data include: 1) acute oral toxicity LD<sub>50</sub> data in rats; 2) laxative activity; digestion, absorption and excretion; 4) subchronic, 90 day, toxicity in dogs; 5) genetic toxicity/carcinogenicity, both *in vitro* and *in vivo*; 6) reproduction toxicity in rats over 3 generations; 7) digestive and biological tolerance in man; and 8) estimated potential human exposure based on predicted consumption.

At issue is whether or not the GPC product(s) is sufficiently similar in chemical composition to the Lonza product to make it behave identically with respect to the biological evaluation required for GRAS approval. On the basis of the data provided this appears to be the case. In particular, the GPC product is clearly very similar to the Lonza product except for its somewhat higher theoretical molecular, permitting the conclusion that these products are biologically equivalent.

Despite the similarity between the GPC and Lonza product, it is still difficult to envision GRAS approval in the absence of any biological studies on the GPC product. In this reviewer's opinion, an acute oral toxicity (LD<sub>50</sub> in rats) should be included with the GRAS petition being prepared by GPC. Additional studies such as *in vitro* evaluation of cariogenicity (a beneficial activity) and laxative activity in dogs (a possible adverse activity) might be useful in helping to market the GPC product.



Table 1 Properties of HSH Products

Degree of Polymerization	Roquette-Lycasin 80/55*	Lonza-Hystar 6075**	GPC Hydrogenated M180***
DP1	8%	12-15%	0.3-3.3
DP2	50-55	6-9	4.2-8.2
DP3	---	8-11	6.3-10.3
DP4	---	---	4.4-8.4
DP>4	---	69 max.	69-85
DP5	---	---	3.9-7.9
DP6	---	---	9.4-13.4
DP3-6	20-25	---	24-40
DP>6	15-20	---	52-68
DP7	---	---	7.5-11.5
DP8	---	---	1.9-5.9
DP>8	---	---	42.6-50.6
DE	<0.2	<0.2	0.3-0.98
Theor MW	630	600-750	1000

\* Roquette GRAS petition

\*\* Lonza GRAS petition

\*\*\* GPC PCT WO 99/36442- "Reduced Malto-Oligosaccharides"

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**000038**

**Statement of data information and availability**

The information supporting the GRAS determination is available for FDA review and copying or would be sent to FDA on request.

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# Appendix A

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**Appendix A**

**Part I**

**Methods to be used in determining compliance with the specifications**

**000041**

<b>STANDARD ANALYTICAL METHOD</b> <b>Grain Processing Corporation</b> Muscatine, Iowa 52761	<b>Saccharide Profile</b> <b>(HPLC)</b>	<b>Method No.:</b> C-46
Reviewed: <u>04/01/98</u> <del>RJH</del> Revised: <u>04/01/98</u> Supersedes: <u>10/25/95</u>	Written by: <u>RJH</u> Approved by: <u>RMT</u> Approval Auth: <u>46-00-01</u>	Revision: 1 Page 1 of 5

## 1. PURPOSE

To determine the polymer distribution, in degrees of polymerization (DP) units, of maltodextrin corn starch hydrolysates.

## 2. PRINCIPLE

An aqueous solution of maltodextrin is chromatographed by HPLC through a Size Exclusion column separating the individual DP units detected by a Differential Refractive Index Detector.

## 3. SCOPE

This method is applicable to all aqueous soluble maltodextrins.

## 4. EQUIPMENT

The equipment listed below can be substituted with equivalent or higher quality equipment.

- 4.1 High Pressure Liquid Chromatographic system consisting of a solvent delivery unit (pump), a column heater, a refractive index detector, and an autosampler.
- 4.2 An IBM Pentium 75 MHz compatible computer with a 200 Mb hard drive, Perkin-Elmer Turbochrom software, Microsoft Windows Version 3.3 and an HP Laser printer.
- 4.3 HPLC Separation Column: A Phenomenex "Rezex" 4% Silver Oligosaccharide column, 200 x 10 mm .
- 4.4 Top loading balance capable of weighing at least  $200 \pm 0.01$  g.
- 4.5 Autosampler vials and caps, approximately 1-mL volume.
- 4.6 Rainin Automatic pipetter with three replaceable shafts to deliver volumes from 10  $\mu$ L to 10 mL.
- 4.7 Disposable nylon disc filters, 0.45 micron pore size.
- 4.8 Disposable syringes, 5 or 10-mL volumes.
- 4.9 Disposable 22-mL glass Wheaton vials with teflon lined screw caps.

000042

<b>STANDARD ANALYTICAL METHOD</b> <b>Grain Processing Corporation</b> Muscatine, Iowa 52761	<b>Saccharide Profile</b> <b>(HPLC)</b>	<b>Method No.:</b> C-46
Reviewed: 04/01/98 <del>RJH</del> Revised: 04/01/98 Supersedes: 10/25/95	Written by: <del>RJH</del> Approved by: <del>RMT</del> 5/18/98 <del>RJH</del> 5/19/98 Approval Auth: 46-00-01	Revision: 1 Page 2 of 5

## 5. REAGENTS

### MAKE SURE ALL STORED REAGENTS AND SOLUTIONS CONTAIN A DATED LABEL AND NFPA CODE

- 5.1 Distilled Water: With a minimum resistivity of 200,000 ohms-cm (conductivity below 5.0  $\mu$ mho/cm) or water of similar quality.
- 5.2 Barnsted Water: Prepared as 18 Meg ohm by filtering distilled water through a four cartridge system to remove organic and inorganic material. Used as the mobile phase solvent.
- 5.3 Mix Bed Ion Exchange Resin, Mallinckrodt, Amberlite MB-1.
- 5.4 Control Maltodextrin, M-200 (Lot # 9806303).

## 6. PROCEDURE

### 6.1 INSTRUMENT SETUP

Column Temperature	80 $\pm$ 1 $^{\circ}$ C
Mobile Phase Solvent	High Purity Water
Pump Flow Rate	0.3 mL/min
Injection Volume	50 $\mu$ L
Detector Sensitivity	16
Detector Cell Temperature	40 $\pm$ 1 $^{\circ}$ C

### 6.2 SAMPLE PREPARATION PROCEDURE

- 6.2.a Weigh approximately 0.1 g of the sample or control into a 22-mL vial and pipet 10 mL of high purity water. Mix well to dissolve. Some samples might require warming to form a homogeneous solution.
- 6.2.b Add approximately 0.2 g of the MB-1 ion exchange resin. Shake well for 30 to 60 seconds and immediately filter the sample through a 0.45 micron nylon disc filter into another 22-mL vial. This will provide additional prepared sample in case further analysis is required. **See NOTE 9.1.**
- 6.2.c Transfer approximately 1 mL of the prepared sample into an autosampler vial and secure with the designated cap.
- 6.2.d Each sample should be followed by a water rinse to flush the fixed loop prior to the injection of the next sample.

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<b>STANDARD ANALYTICAL METHOD</b> Grain Processing Corporation Muscatine, Iowa 52761	<b>Saccharide Profile (HPLC)</b>	<b>Method No.:</b> C-46
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### 6.3 COMPUTER SETUP

6.3.a Setup a "Schedule" listing the sample file names, method and report file, and dilution factor.

6.3.b Make sure the method is up to date with the most current retention time data for each DP unit.

## 7. CALCULATIONS

The computer will calculate the area percent of each peak based on the total area observed in the chromatogram during the designated integration time.

## 8. VALIDATION

### 8.1 Accuracy

An M200 and M180 product were prepared separately at exactly 1% solids for the HPLC saccharide distribution chromatography. A third sample was also prepared as a combined sample containing exactly 1% of each of the same M200 and M180 products. The total DP recovery for the combined sample was compared to the sum of the individual chromatographic runs to obtain the following accuracy data.

Peak Identification	Individually Summed Peak Area Percents	Combined Peak Area Percents	% Recovery (Accuracy)
Highers	45.40	45.66	100.57
DP11	0.11	0.10	90.91
DP10	0.26	0.26	100.00
DP9	0.79	0.79	100.00
DP8	4.09	4.08	99.75
DP7	9.82	9.85	100.30
DP6	11.22	11.27	100.45
DP5	5.73	5.74	100.17
DP4	6.35	6.32	99.53
DP3	8.37	8.28	98.92
DP2	6.25	6.09	97.44
DP1	1.61	1.50	93.17



<b>STANDARD ANALYTICAL METHOD</b> Grain Processing Corporation Muscatine, Iowa 52761	<b>Saccharide Profile</b> (HPLC)	<b>Method No.:</b> C-46
Reviewed: 04/01/98 RJH <del>PK</del> Revised: 04/01/98 Supersedes: 10/25/95	Written by: RJH <del>2</del> Approved by: RMT <del>2</del> 5/18/98 RJH <del>2</del> 5/19/98 Approval Auth: 46-00-01	Revision: 1 Page 4 of 5

## 8.2 Precision

A MALTRIN® control sample (lot # M9806303) was chromatographed thirteen times over a two month period. The individual peak area percentages were averaged for these chromatographic runs. The following table presents the precision data from these chromatographic runs.

Peak Identification	Mean Peak Area %	Std. Dev.(%)	% RSD	Minimum Peak Area %	Maximum Peak Area %
Highers	43.26	0.59	1.37	41.78	43.97
DP10	0.18	0.04	23.47	0.08	0.22
DP9	0.60	0.08	14.10	0.40	0.69
DP8	3.78	0.04	1.15	3.69	3.81
DP7	9.98	0.08	0.82	9.84	10.14
DP6	12.18	0.12	0.98	12.05	12.43
DP5	5.97	0.09	1.47	5.86	6.11
DP4	6.58	0.08	1.24	6.49	6.71
DP3	8.76	0.13	1.45	8.63	9.03
DP2	6.54	0.16	2.38	6.27	6.83
DP1	1.81	0.15	8.06	1.59	2.05

## 9. NOTES

- 9.1 Due to the strong cation and anion resins in the MB-1 mixture, it is recommended that no more than 0.2 g of the resin be added to the sample at one time. It was determined that larger amounts of resin with prolonged residence time with the sample caused the polysaccharide to hydrolyze. It is recommended that the sample solution be filtered after only one minute residence time of shaking the sample.

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<b><u>STANDARD ANALYTICAL METHOD</u></b> <b>Grain Processing Corporation</b> <b>Muscatine, Iowa 52761</b>	<b>Saccharide Profile</b> <b>(HPLC)</b>	<b><u>Method No.:</u></b> <b>C-46</b>
Reviewed: <u>04/01/98</u> <u>RJH</u> Revised: <u>04/01/98</u> Supersedes: <u>10/25/95</u>	Written by: <u>RJH</u> Approved by: <u>RMT</u> <u>RJH</u> Approval Auth: <u>46-00-01</u>	Revision: 1 Page 5 of 5

## 10. REFERENCES

- 10.1 "Estimation of Dextrose Equivalent Value of Starch Hydrolysates from Liquid Chromatographic Profiles", by D. Kiser and R. Hagy, Liquid Chromatographic Analysis of Food and Beverages; Volume 2, 1979.
- 10.2 Phenomenex Bulletin T0103-G-01.
- 10.3 Analytical Development Laboratory, Lab 23, PC, Windows Excel Data file DP.xls.

*The information herein is presented in good faith, but no warranty, expressed or implied, is given nor is freedom from any patent owned by Grain Processing Corporation or by others to be inferred. This analytical procedure is expected to give results of sufficient accuracy for its intended purpose when used by a qualified analyst. Each user, however, is cautioned to confirm the applicability of this procedure to any specific class of samples and to validate the reliability of his own techniques, equipment and standards by appropriate testing. The use of generally accepted good and safe laboratory practices is implied with this method.*

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<b>STANDARD ANALYTICAL METHOD</b> <b>Grain Processing Corporation</b> <b>Muscatine, Iowa 52761</b>	<b>Dextrose Equivalent</b> <b>(Schoorl Method)</b>	<b>Method No.:</b>  C-21 Cust.
Reviewed: 06/19/98 <del>JLW</del> Revised: 06/19/98 Supersedes: New	Written by: CRH Approved by: RMT RJH Approval Auth: 46-00-01	Revision: 5 Page 1 of 9

# 1. PURPOSE

To determine the dextrose equivalent of starch hydrolysates.

# 2. PRINCIPLE

Sugars containing reducing groups are boiled with cupric sulfate-potassium tartrate solutions under alkaline conditions. The cupric ion is reduced to cuprous ion and an oxide is formed. By using a standardized cupric-tartrate solution, the quantity of reducing sugars present can be calculated. In this assay, the copper solution is standardized with dextrose and results expressed as dextrose equivalent.

# 3. SCOPE

This method is used to determine the quantity of reducing sugars (expressed as dextrose) which can then be used to calculate the dextrose equivalent of maltodextrin, corn syrup solids, and related products.

# 4. EQUIPMENT

The equipment listed below may be substituted with similar or equivalent equipment.

- 4.1 Hot Plate.
- 4.2 Volumetric pipettes (assorted sizes).
- 4.3 100-mL beakers without pour spouts.
- 4.4 Water bath with cold tap water.
- 4.5 Glass beads.
- 4.6 Analytical balance capable of weighing 200 g accurately to  $\pm 0.0001$  g.
- 4.7 Electronic top loading balance capable of weighing 2000 g  $\pm 0.1$  g.
- 4.8 DL 25 titrator
- 4.9 Redox electrode, Mettler-Toledo DM140-SC.

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<b>STANDARD ANALYTICAL METHOD</b> Grain Processing Corporation Muscatine, Iowa 52761	<b>Dextrose Equivalent (Schoorl Method)</b>	<b>Method No.:</b> C-21 Cust.
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## 5. REAGENTS

### **MAKE SURE ALL STORED REAGENTS AND SOLUTIONS CONTAIN A DATED LABEL AND NFPA CODE.**

- 5.1 Reverse Osmosis (RO) water: With minimum resistivity of 200,000 ohms-cm (conductivity below 5.0  $\mu$ mhos/cm) or water of similar quality.
- 5.2 Fehling Copper solution A is purchased as a premade solution.
- 5.3 Fehling Alkaline solution B is purchased as a premade solution.
- 5.4 Potassium Iodide (KI), free flowing, ACS grade.
- 5.5 Potassium Iodide Solution : 300  $\pm$  0.1 g of potassium iodide are dissolved in approximately 500 mL of RO water and diluted to volume in a one liter volumetric flask.
- 5.6 Concentrated Sulfuric Acid (H<sub>2</sub>SO<sub>4</sub>), nitrogen free, ACS grade.
- 5.7 Diluted Sulfuric Acid, 6N (approximately) : Add 336  $\pm$  1 mL of concentrated sulfuric acid to approximately 1500 mL RO water. Allow to cool to room temperature and dilute to 2000 mL with RO water. **CAUTION: Always add acid to water when diluting.**
- 5.8 0.1N Sodium Thiosulfate solution - Purchased as certified volumetric solution (0.1  $\pm$  0.0005N).
- 5.9 D-Glucose (Dextrose) - NIST -- Standard Reference Material. Dry at 105°C for four hours and place in desiccator with fresh desiccant.

## 6. PROCEDURE

### **STANDARDIZATION OF COPPER-TARTRATE SOLUTION**

- 6.1 Weigh 1.0000  $\pm$  0.0001 g of oven dried dextrose, transfer quantitatively to a 100-mL volumetric flask, and dilute to volume with RO water.
- 6.2 Prepare samples for the standard curve by adding the following amounts of the Standard Dextrose solution and water to each of twelve separate 100-mL beakers listed in Table 1.

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<b>STANDARD ANALYTICAL METHOD</b> <b>Grain Processing Corporation</b> <b>Muscataine, Iowa 52761</b>	<b>Dextrose Equivalent</b> <b>(Schoorl Method)</b>	<b>Method No.:</b> <b>C-21</b> <b>Cust.</b>
Reviewed: <u>06/19/98</u> <del>JLW</del> Revised: <u>06/19/98</u> Supersedes: <u>New</u>	Written by: <u>CRH</u> Approved by: <u>RMT</u> <del>6/22/98</del> <u>RJH</u> <del>6/23/98</del> Approval Auth: <u>46-00-61</u>	Revision: 5 Page <u>3</u> of <u>9</u>

Table 1

Beaker Number	mL of Std. Dextrose Solution	mL H <sub>2</sub> O
1	1.0	9
2	2.0	8
3	3.0	7
4	3.0	7
5	4.0	6
6	4.0	6
7	5.0	5
8	5.0	5
9	6.0	4
10	7.0	3
11	0.0	10.0
12	0.0	10.0

- 6.3 Accurately add  $10.0 \pm 0.1$  mL of Fehlings Solution A into each beaker.
- 6.4 Accurately add  $10.0 \pm 0.1$  mL of Fehlings Solution B into each beaker.
- 6.5 Swirl each beaker to mix thoroughly.
- 6.6 Place several glass beads into each beaker.
- 6.7 Heat each beaker on a hot plate until the solution boils for 3-4 minutes.
- 6.8 Remove each beaker from the hot plate and cool in a cold tap water bath.
- 6.9 After cooling, add  $10.0 \pm 0.1$  mL KI solution and  $10.0 \pm 0.1$  mL of H<sub>2</sub>SO<sub>4</sub> solution and swirl. **Chemicals must be added in this order.**
- 6.10 Titrate each standard dextrose solution with 0.1N sodium thiosulfate solution on the autotitrator to the first inflection point and record the titration volume.

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<b>STANDARD ANALYTICAL METHOD</b> <b>Grain Processing Corporation</b> <b>Muscatine, Iowa 52761</b>	<b>Dextrose Equivalent</b> <b>(Schoorl Method)</b>	<b>Method No.:</b> <b>C-21</b> <b>Cust.</b>
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**SAMPLE ASSAY- AUTOTITRATION**  
**(See Appendix A for details of Manual Analysis)**

- 6.11 Place a 100-mL beaker on the analytical balance and zero the balance.
- 6.12 Check Table 2 for the approximate sample weight based upon the estimated DE range. Weigh the sample into the beaker and record the weight accurately to 4 decimal places.

Table 2

Sample	DE Range	Approximate Weight *(g)
M-040; M-050	4-7	0.9-1.2
M-100	9-12	0.4-0.54
M-150	13-17	0.28-0.38
M-180	16.5-19.5	0.25-0.35
M-200	20-23	0.22-0.30
M-250	23-27	0.18-0.25

\*The sample weights are adjusted to maintain  $\Delta T$  (See Section 7) in the range of 8-18 mL. The percent solids for samples can be determined by Refractive Index (C-13), Oven Moisture (C-27), or Moisture by Technicon (C-39) and used in the calculation for DE.

- 6.13 Add enough RO water to bring total volume to approximately 10 mL.
- 6.14 Accurately add 10 mL of Fehlings Solution A into each sample beaker.
- 6.15 Accurately add 10 mL of Fehlings Solution B into each sample flask.
- 6.16 Swirl each beaker to mix thoroughly.
- 6.17 Place several glass beads into each beaker.
- 6.18 Heat each sample beaker on a hot plate until the solution boils for 3-4 minutes.
- 6.19 Remove each beaker from the hot plate and cool in a cold tap water bath.
- 6.20 After cooling, add  $10.0 \pm 0.1$  mL of the KI solution and  $10.0 \pm 0.1$  mL of the  $H_2SO_4$  solution and swirl. **Chemicals must be added in this order.**

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<b>STANDARD ANALYTICAL METHOD</b> Grain Processing Corporation Muscatine, Iowa 52761	<b>Dextrose Equivalent</b> (Schoorl Method)	<b>Method No.:</b> C-21 Cust.
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6.21 Place each sample beaker into the DL25 titrator. Using a redox electrode titrate each sample with 0.1N sodium thiosulfate to the first inflection point. The DL25 titrator will calculate the percent reducing sugars for each sample. See the CALCULATIONS section (7) for the autotitrator equation to calculate the DE.

## 7. CALCULATIONS

### STANDARDIZATION CALCULATION

7.1  $\Delta T(\text{STANDARD}) = \text{mL of 0.1N Sodium Thiosulfate to titrate the blank minus the mL of Sodium Thiosulfate to titrate the dextrose solution.}$

7.2  $\text{Ratio Factor} = \Delta T(\text{STANDARD}) / \text{mL of Standard Dextrose Solution.}$

7.3  $\text{Average Ratio Factor} = \text{Average of the Ratio Factors for all levels of the Standard Dextrose Solution.}$

7.4  $\text{Concentration Factor(CF)} = \text{mg dextrose per mL of Standard Dextrose Solution} / \text{Average Ratio Factor.}$

### EXAMPLE:

Standard Dextrose Solution (mL)	Volume of 0.1N Sodium Thiosulfate (mL)	$\Delta T$ (Standard)	Ratio Factor
0(Blank)	26.3		
0(Blank)	26.3		
1	23.3	3.0	3.00
2	20.4	5.9	2.95
3	17.1	9.2	3.07
3	17.1	9.2	3.07
4	14.1	12.2	3.05
4	14.1	12.2	3.05
5	11.2	15.1	3.02
5	11.2	15.1	3.02
6	8.7	17.6	2.93
7	5.1	21.2	3.03

<b>STANDARD ANALYTICAL METHOD</b> Grain Processing Corporation Muscatine, Iowa 52761	<b>Dextrose Equivalent</b> <b>(Schoorl Method)</b>	<b>Method No.:</b> C-21 Cust.
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The Average Ratio Factor = 3.02

Standard Dextrose Solution = 1.000 g/100 mL  
 = 1000 mg/100 mL  
 = 10 mg/mL

CF = 10 mg dextrose/3.02 mL = 3.311 mg/mL

#### AUTOTITRATION : Calculation of DE in sample

7.5  $\Delta T(\text{SAMPLE})$  = mL of 0.1N Sodium Thiosulfate to titrate the blank minus the mL of Sodium Thiosulfate to titrate the sample.

7.6 Unit Conversion factor(UCF) = 1000 mg/g.

7.7 Solids = Conversion of % Solids to the decimal value  
 = % Solids/100

7.8 Dextrose Equivalence (DE) Equations

$$D.E. = \frac{\Delta T(\text{Sample}) \times CF}{\text{Sample Wt. (g)} \times UCF \times \text{Solids}} \times 100$$

$$DE = \frac{\% \text{ REDUCING SUGARS}}{\% \text{ SOLIDS}} \times 100$$

$$= \frac{\Delta T(\text{Sample}) \times CF}{\text{Sample Wt. (g)} \times 10 \times \text{Solids}}$$

Example: Blank Titer = 27.8 mL, Sample Titer = 17.6 mL,  
 Sample Wt. = 1.0525 g, % Solids = 95.5

$\Delta T$  = Blank - Sample = 27.8 mL - 17.6 mL = 10.2 mL  
 Solids = % Solids/100 = 95.50/100 = 0.9550

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<b>STANDARD ANALYTICAL METHOD</b> <b>Grain Processing Corporation</b> <b>Muscataine, Iowa 52761</b>	<b>Dextrose Equivalent</b> <b>(Schoorl Method)</b>	<b>Method No.:</b>  C-21 Cust.
Reviewed: 06/19/98 <del>JLW</del> Revised: 06/19/98 Supersedes: New	Written by: CRH Approved by: RMT 6/22/98 RJM 6/25/98 Approval Auth: 46-00-61	Revision: 5 Page 7 of 9

$$D.E. = \frac{10.2 \text{ ML} \times 3.322 \text{ MG/ML}}{1.0525 \text{ G} \times 10 \times 0.9550} = 3.37 \text{ DE}$$

## 8. VALIDATION

### 8.1 Accuracy

The accuracy was not determined.

### 8.2 Precision

Not available.

## 9. REFERENCES

- 9.1 U.S. Pharmacopoeia National Formulary, Sixteenth Edition, 1985, Test Solutions, pg. 1423
- 9.2 Corn Industries Research Foundation, Sixth Edition, Procedure D-52.
- 9.3 Standard Analytical Methods of Grain Processing Corporation, 4/1/70.

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<b>STANDARD ANALYTICAL METHOD</b> <b>Grain Processing Corporation</b> <b>Muscatine, Iowa 52761</b>	<b>Dextrose Equivalent</b> <b>(Schoorl Method)</b>	<b>Method No.:</b> <b>C-21</b> <b>Cust.</b>
Reviewed: <u>06/19/98</u> <del>JLW</del> Revised: <u>06/19/98</u> Supersedes: <u>New</u>	Written by: <del>CRH</del> Approved by: <del>RMT</del> <u>6/22/98</u> <u>RJH</u> <u>6/25/98</u> Approval Auth: <u>46-00-01</u>	Revision: 5 Page <u>8</u> of <u>9</u>

## APPENDIX A -MANUAL TITRATION

### 4. EQUIPMENT

**The equipment listed below may be substituted with similar or equivalent equipment.**

- 4.1 Erlenmeyer flasks, 200 or 250-mL
- 4.2 Burette, 50-mL, graduated with 0.1 mL divisions
- 4.3 Lead collars for Erlenmeyer flasks
- 4.4 Tuttle caps for Erlenmeyer flasks

### 5. REAGENTS

**MAKE SURE ALL STORED REAGENTS AND SOLUTIONS CONTAIN A DATED LABEL AND NFPA CODE.**

- 5.1 Cupric Sulfate ,  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , ACS grade.
- 5.2 Fehling Solution A: Schoorl A:  $1108.5 \pm 0.1$  g of cupric sulfate are dissolved in RO water and diluted to sixteen liters.
- 5.3 Sodium Hydroxide, NaOH, pellets (Nitrogen free), ACS grade.
- 5.4 Potassium sodium tartrate,  $\text{KNaC}_4\text{H}_4\text{O}_6 \cdot 4\text{H}_2\text{O}$ , Rochelle Salt, ACS grade.
- 5.5 Fehling Solution B: Schoorl B:  $5536 \pm 1$  g of Rochelle salt and  $1600 \pm 1$  g of sodium hydroxide are dissolved in RO water and diluted to sixteen liters.
- 5.6 Litner starch.
- 5.7 1% Starch Indicator Solution: 10 g of soluble starch (Litner) is dissolved by boiling 3-5 minutes in approximately 100 mL of RO water, air cooled and diluted to 1000 mL with RO water in a one liter volumetric flask.

## **SAMPLE ASSAY- MANUAL TITRATION**

- 6.11 Place a 200 or 250-mL flask on the analytical balance and zero the balance.

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<b>STANDARD ANALYTICAL METHOD</b> <b>Grain Processing Corporation</b> Muscatine, Iowa 52761	<b>Dextrose Equivalent</b> <b>(Schoorl Method)</b>	<b>Method No.:</b> C-21 Cust.
Reviewed: 06/19/98 JLW <del>XXXX</del> Revised: 06/19/98 Supersedes: New	Written by: CRH <del>XXXX</del> Approved by: RMT <del>XXXX</del> 6/22/98 RJH <del>XXXX</del> 6/25/98 Approval Auth: 46-00-01	Revision: 5 Page 9 of 9

- 6.12 Check Table 2 for the approximate sample weight based upon the estimated DE range. Weigh the sample into the flask and record the weight accurately to 4 decimal places.

Table 2

Sample	DE Range	Approximate Weight *(g)
M-040; M-050	4-7	0.9-1.2
M-100	9-12	0.4-0.54
M-150	13-17	0.28-0.38
M-180	16.5-19.5	0.25-0.35
M-200	20-23	0.22-0.30
M-250	23-27	0.18-0.25

\*The sample weights are adjusted to maintain  $\Delta T$  (See Section 7) in the range of 8-18 mL. The percent solids for samples can be determined by Refractive Index (C-13), Oven Moisture (C-27), or Moisture by Technicon (C-39) and used in the calculation for DE.

- 6.13 Add enough RO water to bring total volume to approximately 30 mL.
- 6.14 Accurately add 10.0 mL of Fehlings Solution A into each sample flask.
- 6.15 Accurately add 10.0 mL of Fehlings Solution B into each sample flask.
- 6.16 A blank containing approximately 30 mL of RO water plus  $10.0 \pm 0.1$  mL of Fehlings Solution A and  $10.0 \pm 0.1$  mL of Fehlings Solution B is to be assayed at the beginning of each shift.
- 6.17 Swirl each flask to mix thoroughly.
- 6.18 Place several glass beads into each flask, cover with a Tuttle cap, and place a lead collar around the neck of each flask.
- 6.19 Heat each sample flask on a hot plate until the solution boils for 3-4 minutes.
- 6.20 Remove each flask from the hot plate and cool in a cold tap water bath.
- 6.21 After cooling, add  $10.0 \pm 0.1$  mL of the KI solution and  $10.0 \pm 0.1$  mL of the  $H_2SO_4$  solution and swirl. Chemicals must be added in this order.
- 6.22 Titrate each sample with 0.1N sodium thiosulfate solution until a light straw color is reached. Add a small amount of starch indicator and titrate until sample turns a milky white, Record the titration volume.

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<b>STANDARD ANALYTICAL METHOD</b> <b>Grain Processing Corporation</b> <b>Muscataine, Iowa 52761</b>	<b>Oven Moisture &amp; Solids</b>	<b>Method No.:</b> <b>C-27</b>
Reviewed: <u>01/11/99</u> <del>CLS</del> Revised: <u>01/11/99</u> Supersedes: <u>10/13/95</u>	Written by: <u>DLM</u> Approved by: <u>KGL</u> <del>1/20/99</del> <u>RJH</u> <del>1/22/99</del> Approval Auth: <u>46-00-01</u>	Revision: 2 Page <u>1</u> of 4

# 1. PURPOSE

To determine the moisture and/or solids content of maltodextrins and corn syrup solids.

# 2. PRINCIPLE

It is possible to remove volatiles from materials by drying them in an oven at 105 °C for 4 hours. These volatiles are referred to as the moisture content of the sample.

# 3. SCOPE

This method is used to determine the amount of moisture and/or solids present in maltodextrins, corn syrup solids, and related products.

# 4. EQUIPMENT

The equipment listed below can be substituted with equal or higher quality equipment.

- 4.1 Aluminum weighing pans with lids.
- 4.2 Analytical balance capable of weighing 100 g with an accuracy of  $\pm 0.0001$  g.
- 4.3 An electrical oven (convection or forced air) capable of maintaining a temperature of  $105 \pm 2$  °C.
- 4.4 Desiccator with fresh dessicant.
- 4.5 Tongs.

# 5. REAGENTS

None.

# 6. PROCEDURE

- 6.1 Predry the weighing pans for a minimum of 1 hour in an oven at the drying temperature of 105 °C and place in a desiccator prior to use. Allow the weighing pans to equilibrate to room temperature.
- 6.2 Zero the balance.
- 6.3 Weigh the sample pan and lid to  $\pm 0.0001$  g and record. Always be sure to use tongs when handling sample dish and lid to avoid any oil that may occur from ones fingers.

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Reviewed: 01/11/99 <del>CLS</del> Revised: 01/11/99 Supersedes: 10/13/95	Written by: <del>DLM</del> Approved by: <del>KGL</del> 11/20/99 RJH 11/22/99 Approval Auth: 46-00-01	Revision: 2 Page 2 of 4

- 6.4 Rezero the balance with the sample pan and lid on it. Add approximately 5 g of sample to the sample pan. Record the sample weight accurately to  $\pm 0.0001$  g.
- 6.5 Using tongs, place the lid under the sample pan and heat in the oven for 4 hours at 105 °C.
- 6.6 Use tongs to place the lid on the sample pan, remove from the oven and place in a cool desiccator for 30 minutes or until the sample and pan have cooled to room temperature..
- 6.7 Zero the balance.
- 6.8 Weigh the sample pan, lid, and dried sample. Record the weight accurately to  $\pm 0.0001$  g.

## 7. CALCULATIONS

### 7.1

- A = Weight of sample pan, lid, and dry sample in grams.  
B = Weight of sample pan and lid in grams.  
C = Weight of the sample before drying grams.

$$\% \text{ Solids} = \frac{(A-B)}{C} \times 100$$

### 7.2

$$\% \text{ Solids} = \frac{\text{Dried Sample (g)}}{\text{Sample Weight (g)}} \times 100$$

### 7.3

$$\% \text{ Moisture} = 100\% - \% \text{ Solids}$$

### Example:

- A = 21.6867 g  
B = 16.8324 g  
C = 5.0796 g

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Reviewed: 01/11/99 <del>CLS</del> Revised: 01/11/99 Supersedes: 10/13/95	Written by: DLM Approved by: KGL <del>1/20/99</del> RJH <del>1/22/99</del> Approval Auth: 46-00-01	Revision: 2 Page 3 of 4

$$\% \text{ Solids} = \frac{21.6867 \text{ g} - 16.8324 \text{ g}}{5.0796 \text{ g}} \times 100$$

$$\% \text{ Solids} = \frac{4.8543 \text{ g}}{5.0796 \text{ g}} \times 100 = 95.56\%$$

$$\% \text{ Moisture} = (100.00\% - 95.56\%) = 4.44\%$$

## 8. VALIDATION

### 8.1 Accuracy.

Not available.

### 8.2 Precision

A maltodextrin was assayed using four replicate samples of the same product to determine the repeatability or precision of the method. The following table summarizes this data:

**% Solids Precision Data**

Mean % Solids	Standard Deviation (%)	% RSD	Precision at 95% Confidence
95.06	± 0.06	0.06	± 0.12%

## 9. REFERENCES

- 9.1 Official methods of Analysis - AOAC, Fourteenth Edition, Procedure 31.006, Page 574, 1984.
- 9.2 U.S. Pharmacopeia National Formulary, Sixteenth Edition, Procedure 731, Page 1249 and Page 1612, 1985.
- 9.3 Standard Analytical Methods of Grain Processing Corporation, 4/1/70.
- 9.4 American Association of Cereal Chemists, Eighth Edition, Procedure 44-20, March 1983.

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<b>STANDARD ANALYTICAL METHOD</b> <b>Grain Processing Corporation</b> <b>Muscataine, Iowa 52761</b>	<b>Ash (non-sulfated)</b>	<b>Method No.</b> <b>C-49</b>
Reviewed: 09/26/96 <del>JLW</del> Revised: 09/23/96 Supersedes: <u>New</u>	Written by: <del>JLW</del> Approved by: <del>RMT</del> 10/22/96 <del>RHM</del> 10/28/96 Approval Auth: 46-00-01	Revision: 0 Page 1 of 4

# 1. PURPOSE

To determine the amount of inorganic material present in starch hydrolysates.

# 2. PRINCIPLE

Inorganic residues present in starch hydrolysates remain after the sample has been ignited in a muffle furnace..

# 3. SCOPE

Applicable to all maltodextrins, corn syrup solids and related products.

# 4. EQUIPMENT

- 4.1 Silica evaporating dishes (Vycor), 100 to 200-mL capacity.
- 4.2 Muffle furnace capable of maintaining a temperature of  $550 \pm 25$  °C.
- 4.3 Analytical Balance capable of weighing  $200 \pm 0.0001$  g.
- 4.4 Desiccator with fresh dry desiccant (Drierite®).
- 4.5 Metal Tongs.
- 4.6 Hot plate.

# 5. REAGENTS

- 5.1 None required

# 6. PROCEDURE

- 6.1 Each Vycor dish should be ignited in 550 °C muffle furnace for at least one hour and cooled in a desiccator for one hour before using.
- 6.2 Zero the analytical balance.
- 6.3 Always use tongs when handling the Vycor dishes. (See Note 9.1) Weigh each dish accurately to 0.0001 g and record the weight.

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STANDARD ANALYTICAL METHOD Grain Processing Corporation Muscatine, Iowa 52761	Ash (non-sulfated)	Method No. C-49
Reviewed: 09/26/96 JLV <del>10/22/96</del> Revised: 09/23/96 Supersedes: <u>New</u>	Written by: JLV <del>10/22/96</del> Approved by: RMT <del>10/22/96</del> RIH <del>10/22/96</del> Approval Auth: 46-00-01	Revision: 0 Page 2 of 4

- 6.4 Add approximately 5 grams of sample to each dish and accurately record the weight of the dish and sample to  $\pm 0.0001$  g.
- 6.5 Place dish in a muffle furnace at  $550 \pm 25$  °C for at least 2 hours or until ash is free from carbon.
- If any black color remains in the sample, return it to the furnace for at least two hours further firing.
  - If color still remains, cool the sample and carefully wet it with water or dilute HCl and return it to the furnace for at least 2 hours.
- 6.6 Remove the samples from the furnace and cool completely in a desiccator for one hour. Do not close the desiccator lid tightly until the dishes have cooled for 5-10 minutes.
- 6.7 Zero the analytical balance.
- 6.8 Weigh each Vycor dish containing the ash to 4 decimal places.

## 7. CALCULATIONS

$$\text{Percent Ash (as is)} = \frac{\text{Ash weight (g)}}{\text{Sample weight (g)}} \times 100$$

### EXAMPLE CALCULATION

#### Before Firing:

Weight of Sample and dish = 87.7492 g  
 Weight of dish = 82.7008 g  
 Weight of Sample = 5.0484 g

#### After Firing:

Weight of Ash and dish = 82.7151 g  
 Weight of dish = 82.7008 g  
 Weight of Ash = 0.0143 g

$$\% \text{ Ash} = \frac{0.0143\text{g}}{5.0484\text{g}} \times 100 = 0.28\%$$

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## 8. VALIDATION

### 8.1 Accuracy

Not determined.

### 8.2 Precision

The precision was evaluated by assaying a control sample eight times. The results of this testing are summarized below:

Mean % Ash	Std Dev (%)	% RSD
0.243	0.012	4.80

### 8.3 Correlation

#### Correlation to USP procedure Residue on Ignition (Sulfated Ash)

A correlation study was conducted by assaying 121 maltodextrin and corn syrup solid samples by using both a sulfated ash and a non-sulfated ash method. The results are summarized below.

	Ave. Ash Value (%)	Std. Dev.
Sulfated Ash	0.324	0.065
Non-Sulfated Ash	0.276	0.067

To convert a non-sulfated ash to a sulfated ash value add 0.048 to the non-sulfated value.

## 9. NOTES AND PRECAUTIONS

9.1 Wear the appropriate laboratory safety equipment.

## 10. REFERENCES

10.1 U. S. Pharmacopeia National Formulary, Procedure 281, p. 1192.

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STANDARD ANALYTICAL METHOD Grain Processing Corporation Muscatine, Iowa 52761	Ash (non-sulfated)	Method No. C-49
Reviewed: 09/26/96 <del>ILW</del> Revised: 09/23/96 Supersedes: <u>New</u>	Written by: <del>ILW</del> Approved by: <del>RMT</del> 10/22/96 <del>RIH</del> 10/28/96 Approval Auth: 46-00-01	Revision: 0 Page 4 of 4

10.2 Official Methods of Analysis, AOAC, Fourteenth Edition, Procedure 14.006, p. 249, 1984.

10.3 Corn Industries Research Foundation, Sixth Edition, Procedure R-6.

10.4 GPC Resarch Notebook 1625, pp 14-17.

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<b>STANDARD ANALYTICAL METHOD</b> <b>Grain Processing Corporation</b> <b>Muscataine, Iowa 52761</b>	<p style="text-align: center;">pH</p>	<b>Method No.:</b> <p style="text-align: center;">C-25</p>
Reviewed: <u>12/09/98</u> <del>CLS</del> Revised: <u>12/09/98</u> Supersedes: <u>10/13/95</u>	Written by: <u>DJS</u> Approved by: <u>KGL</u> <del>1/20/99</del> <u>RJH</u> <del>1/22/99</del> Approval Auth: 46-00-01	Revision: 3 Page <u>1</u> of <u>3</u>

# 1. PURPOSE

To determine the pH of an aqueous starch hydrolysate.

# 2. PRINCIPLE

pH is a measure of the chemical potential of the solution as related to the concentration of the hydrogen ions in that solution. The pH value is normally determined by measuring the potential difference between a hydrogen sensing glass electrode and a standard reference electrode immersed in a solution of sample.

# 3. SCOPE

This method is applicable to the measurement of pH in aqueous solutions of maltodextrin, corn syrup solids, and related products.

# 4. EQUIPMENT

The equipment below can be substituted with equal or higher quality equipment.

- 4.1 Digital pH meter capable of measuring pH values to 0.01 pH units.
- 4.2 Electrode; with combined or separate standard reference electrode (calomel or silver/silver chloride are preferred).
- 4.3 Magnetic stirrer and stirring magnet.

# 5. REAGENTS

- 5.1 Reverse Osmosis (RO)/Ion Exchanged Water – With a minimum resistivity of 200,000 ohms-cm (conductivity below 5.00  $\mu$ mhos/cm) or water of similar quality.
- 5.2 pH Standard Buffers, Commercial; pH 4.0, 7.0, and 10.0 with an accuracy of 0.01 pH units.

# 6. PROCEDURE

## FOR STANDARDIZING THE pH METER

(Should be done at least once at the beginning of each shift)

- 6.1 Place the electrode in a "fresh" standard buffer with a known pH value of about 7.0, and stir at a rate sufficient to produce a small vortex at the solution surface (Note 9.1).
- 6.2 Switch meter to the pH mode and allow the meter to stabilize before pushing the "CALIBRATE" button.

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<b>STANDARD ANALYTICAL METHOD</b> Grain Processing Corporation Muscatine, Iowa 52761	<p style="text-align: center;">pH</p>	Method No.: <p style="text-align: center;">C-25</p>
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- 6.3 Remove electrode from the pH 7 buffer, rinse with warm tap water, and remove the excess by blotting with a clean, dry tissue.
- 6.4 Place the electrode in the second standard buffer, either pH 4.0 or pH 10.0, and stir to produce a small vortex at the solution surface (Note 9.2).
- 6.5 Remove the electrode from the second buffer, rinse with warm tap water, and remove the excess by blotting with a clean and dry tissue prior to sample analysis.
- 6.6 Store the electrode in fresh pH 4 buffer when not in use. **Do not store in water!**

### **PROCEDURE FOR SAMPLE ASSAYS**

- 6.7 Prepare a 20% wt/wt solution by dissolving  $50.0 \pm 0.1$  g of sample (as is - see Note 9.3) in approximately 200.0 g of RO water for a final total weight of 250.0 g. This should be done by dissolving the 50 g of sample in approximately 150 g water and then add the remaining water by rinsing down the sides of the container. Stir this solution at a rate sufficient to produce a small vortex at the liquid surface.
- 6.8 Immerse the standardized electrode in the sample. Observe and record the pH value to the nearest 0.01 pH unit, after a stable reading is achieved. If erratic and/or unstable readings are observed, refer to the pH meter manufacturer's instruction manual.

## **7. VALIDATION**

### **7.1 Accuracy**

The accuracy is dependent upon the calibration with two commercial standard buffer solutions of different pH values. Most commercial pH electrodes have an accuracy no better than  $\pm 0.05$  pH units.

### **7.2 Precision**

The precision of pH measurement was determined by making twelve pH determinations on a single MALTRIN® M100 sample.

Mean	=	4.13
Minimum	=	4.10
Maximum	=	4.20
Standard Deviation	=	$\pm 0.05$

## **8. REFERENCES**

- 8.1 **Procedure E48, Standard Analytical Methods of the Member Companies of Corn Industries Research Foundation, Sixth Edition**

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<b>STANDARD ANALYTICAL METHOD</b> <b>Grain Processing Corporation</b> Muscatine, Iowa 52761	<b>pH</b>	<b>Method No.:</b> C-25
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9. NOTES AND PRECAUTIONS

- 9.1 To ensure pH electrodes are hydrated, soak overnight in warm tap water or buffer solution.
- 9.2 Select two buffer solutions whose difference in pH does not exceed 4 pH units such that the pH of the test solution falls between the two selected buffers.
- 9.3 Since maltodextrin product moistures are consistently low (4-6%), "as is" weights are used for procedural convenience. Actual solution concentrations may vary slightly.

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<b>STANDARD ANALYTICAL METHOD</b> Grain Processing Corporation Muscatine, Iowa 52761	<b>Heavy Metals</b> (Modified USP Method I)	<b>Method No.:</b> C-37
Reviewed: 05/07/98 RJH <del>SK</del> Revised: 05/07/98 Supersedes: 01/15/96	Written by: RJH Approved by: RMT 6/16/98 RJH 7/30/98 Approval Auth: 46-00-01	Revision: 4 Page 1 of 6

# 1. PURPOSE

To determine if the heavy metals content of maltodextrin and corn syrup solids meets a prescribed specification limit for each final product tested.

# 2. PRINCIPLE

Heavy metals (Ag, As, Bi, Cd, Cu, Hg, Pb, Sb, Sn) are solubilized with dilute nitric acid and then reacted with H<sub>2</sub>S to form an insoluble colored metal sulfide. The resultant turbidity is proportional to the heavy metals concentration.

# 3. SCOPE

This method is applicable for the determination of heavy metals in soluble maltodextrin and corn syrup solids products.

# 4. EQUIPMENT

The equipment listed below can be substituted with similar or equivalent equipment.

- 4.1 Electronic Top Loading Balance; capable of weighing at least 200 g accurately to at least  $\pm 0.01$  g.
- 4.2 100-mL and 1000-mL Volumetric Flasks.
- 4.3 150-mL beakers.
- 4.4 Labline Multi-Stirrer Variable control Stir Plate
- 4.5 pH Paper (pH range 0-13).
- 4.6 pH Paper (pH range 3.0-5.5).
- 4.7 Exax Brand Nessler Color Comparison Tubes (50 mL).
- 4.8 Tube Support for Nessler Color Tubes.
- 4.9 Lecture Size Gas Cylinder of Hydrogen Sulfide Gas (H<sub>2</sub>S); UN1053, CP Grade, with a single stage gas regulator and gas cylinder stand.
- 4.10 Pyrex Brand Gas Washing Bottle (125 mL).
- 4.11 1000-mL and 2000-mL Erlenmeyer Flasks.
- 4.12 Fume Hood.

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- 4.13 Lead free (acid washed plastic) container for storage of 1000 mL of Lead Nitrate stock solution.
- 4.14 Automatic pipetter (Rainin) with three replaceable shafts capable of delivering volumes from 10  $\mu$ L to 10 mL.

## 5. REAGENTS

**MAKE SURE ALL STORED REAGENTS AND SOLUTIONS CONTAIN A DATED LABEL AND NFPA CODE.**

- 5.1 Reverse Osmosis (RO) Water - Having a minimum resistivity of 200,000 ohm-cm (5.0  $\mu$ mhos/cm conductivity) or water of similar quality. Prepare fresh daily as needed.
- 5.2 Concentrated Nitric Acid, HNO<sub>3</sub>, Trace Metal Grade. Store in specified acid storage area.
- 5.3 Nitric Acid, 0.1N: To approximately 500 mL of RO water in a one liter volumetric flask pipet 6.3 mL of concentrated nitric acid, mix, and dilute to volume with RO water.
- 5.6 Ammonium Hydroxide, NH<sub>4</sub>OH, Trace Metal Grade. Store in specified base storage area.
- 5.7 Ammonium Hydroxide, approximately 6N: In a hood, carefully pipet 40 mL of concentrated ammonium hydroxide to a 100-mL volumetric flask containing about 50 mL of RO water. Mix and cool the contents of the flask to room temperature then dilute to volume with RO water.
- 5.8 Glacial Acetic Acid, CH<sub>3</sub>COOH, Trace Metal Grade.
- 5.9 Acetic Acid, approximately 1N: To a 100-mL volumetric flask containing about 50 mL RO water, pipet 6 mL of glacial acetic acid. Mix thoroughly while diluting to volume with RO water.
- 5.10 Lead Nitrate
- 5.10.a. Certified ACS Grade.
- 5.10.b. Lead Reference Standard Solution 1000  $\pm$  10 ppm.
- 5.11 Lead Nitrate Stock Solution, 100 ppm (100  $\mu$ g/mL) as Pb.

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5.11.a. Dissolve 159.8 mg ( $0.1598 \pm 0.0001$  g) of lead nitrate in approximately 100 mL RO water containing approximately 1 mL concentrated nitric acid and dilute to 1000 mL volume with RO water. Transfer to lead free container and store at room temperature. Discard stock solution if it becomes turbid.

5.11.b An alternate method for preparation of a 100 ppm lead nitrate stock solution: Pipet 10.0 mL of Lead Reference Standard Solution (1000 ppm) into a 100-mL volumetric flask and dilute to volume with RO water. Transfer and store this solution as stated in section 5.11.a.

5.12 Lead Nitrate Working Solution - 10 ppm (10  $\mu$ g/mL) as Pb: Using either of the lead nitrate stock solutions (5.11. a or b) pipet 10.0 mL of the 100 ppm solution into a 100-mL volumetric flask and dilute to volume with RO water. Transfer the working solution to a lead free container and store at room temperature. Discard working solution if it becomes turbid.

5.13 Hydrogen Sulfide Gas (H<sub>2</sub>S) -- CP Grade (see Note 9.1).

5.14 Sodium Hypochlorite - Industrial grade "Truck or Raw Hypo" containing approximately 16% active chlorine.

5.15 Saturated Hydrogen Sulfide Solution: **CAUTION: KEEP THIS SOLUTION IN AN OPERATIONAL FUME HOOD.** To prepare this solution, bubble hydrogen sulfide from a Lecture Size Gas cylinder at a moderate rate into a gas washing bottle containing 100 - 250 mL of RO water for 2 minutes. See Note 9.3. The vapor vent tube from the gas washing bottle runs down into a 1000-mL Erlenmeyer trap. This empty container trap protects the hydrogen sulfide saturated solution from possible contamination due to pressure fluctuations. The empty trap vents into the bottom of the 2000-mL Erlenmeyer trap which contains approximately 1500 mL of "Truck or Raw Hypo". A second similar "Hypo containing" trap is setup to handle any overflow from the first trap. The vapor vent tube from this trap vents into an operating fume hood. At this final vent no detectable quantity of hydrogen sulfide should be detected. (Safety Note 9.2.d.)

## 6. PROCEDURE

6.1 Calculate the weight in grams of the substance to be tested by the following formula:

$$\frac{2.00}{1000 \times L}$$

in which L is the Heavy Metals Limit expressed as a percentage.

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If the heavy metals specification limit is 5 ppm or 0.0005%, then the formula would calculate an amount of 4.00 g for the test substance.

- 6.2 Accurately weigh  $4.00 \pm 0.01$  g of test substance into a 150-mL beaker and dissolve the material in approximately 25 mL of 0.1N nitric acid.
- 6.3 Adjust the solution to pH 3.0-4.0 with 1N acetic acid or 6N ammonium hydroxide and test with an appropriate range pH paper.
- 6.4 A heavy metals reference standard is prepared by pipeting 2.0 mL of the 10  $\mu$ g/mL lead nitrate working solution into a 50-mL Nessler color-comparison tube and diluting to approximately 25 mL with RO water. Adjust the pH of the reference solution between 3.0 and 4.0 with a short range pH paper using either 1N acetic acid or 6N ammonium hydroxide. Mix thoroughly.
- 6.5 To each of the Nessler color comparison tubes containing the Standard Reference Solution or the Sample Test Preparation, pipet 10 mL of the saturated hydrogen sulfide solution and dilute to volume with RO water. Cap with stopper and mix thoroughly. (See Note 9.1)
- 6.6 Let the reference and test sample tubes stand for 5 minutes to allow for complete color development. Compare the color of the resulting solutions by viewing down through the length of the Nessler color comparison tube over a white surface. (See Notes 9.2)

## 7. CALCULATIONS

Each test sample is reported as less than or greater than that of the reference solution concentration.

## 8. VALIDATION

### 8.1 Accuracy

The accuracy is dependent upon a subjective judgement of color comparison between a standard and a sample.

### 8.2 Precision

Samples of 5, 8, 10, and 15 ppm lead sulfide standards and a blank were used to evaluate the subjective color comparison. Each sample was treated as an unknown. The evaluation by four untrained individuals resulted in greater than 80% correct color comparisons. The evaluation by a trained technician resulted in 100% correct color evaluation.

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## 9. SAFETY

- 9.1 Store in specified acid storage area.
- 9.2 For the safety of all laboratory personnel in the immediate vicinity during use of hydrogen sulfide certain precautions in handling and confinement are necessary.
  - 9.2.a. Adequately ventilated fume hood.
  - 9.2.b. All equipment in contact with H<sub>2</sub>S must remain in the hood.
  - 9.2.c. Use a containment system for the preparation of an aqueous saturated hydrogen sulfide solution and elimination of excess gas (Sections 5.14-15).
  - 9.2.d. The "truck or raw hypo" must be replaced in the scrubber unit at least once a week or more often depending on the frequency of generation of the hydrogen sulfide saturated solution.
  - 9.2.e. If hydrogen sulfide is detected by its rotten egg smell outside the fume hood, the safety department should immediately be notified and all personnel removed from the contaminated area.

## 10. NOTES

- 10.1 Two or more gas washing bottles can be connected in series if more H<sub>2</sub>S saturated water is needed for sample analyses.
- 10.2 During the ashing procedure, strict control of the muffle furnace temperature at 600 ± 10 °C is imperative so that lead salts do not vaporize (some salts volatilize at around 625 °C).
- 10.3 It is recommended that the standard reference solutions be developed for color first to test the saturated hydrogen sulfide solution prior to running the samples.

## 11. REFERENCES

- 11.1 The United States Pharmacopeia, Twenty Second Edition, Official from January 1, 1990, United States Pharmacopeia Convention, Inc., Rockville, MD, 1989, pages 1523-1524.
- 11.2 GPC Research Notebook: 1673, pp. 1-28.



<b>STANDARD ANALYTICAL METHOD</b> <b>Grain Processing Corporation</b> Muscatine, Iowa 52761	<b>Heavy Metals</b> <b>(Modified USP Method I)</b>	<b>Method No.:</b> C-37
Reviewed: <u>05/07/98</u> <u>RJH</u> Revised: <u>05/07/98</u> Supersedes: <u>01/15/96</u>	Written by: <u>RJH</u> Approved by: <u>RMT</u> <u>RJH</u> Approval Auth: 46-00-01	Revision: 4 Page <u>6</u> of <u>6</u>

*The information herein is presented in good faith, but no warranty, expressed or implied, is given nor is freedom from any patent owned by Grain Processing Corporation or by others to be inferred. This analytical procedure is expected to give results of sufficient accuracy for its intended purpose when used by a qualified analyst. Each user, however, is cautioned to confirm the applicability of this procedure to any specific class of samples and to validate the reliability of his own techniques, equipment and standards by appropriate testing. The use of generally accepted laboratory safety practices are implied with this method.*

000072

<b>STANDARD ANALYTICAL METHOD</b> <b>Grain Processing Corporation</b> <b>Muscataine, Iowa 52761</b>	<b>Anion Screening Assay</b>	<b>Method No.</b> <b>C-56</b>
Reviewed: 11/06/97 RJH <del>11/12/97</del> Revised: 11/06/97 Supersedes: New	Written by: RJH <del>11/12/97</del> Approved by: RMT <del>11/12/97</del> RJH <del>11/12/97</del> Approval Auth: 46-00-01	Revision: 0 Page 1 of 9

## 1. PURPOSE

The Anion Screen method is used to quantitate the concentration of aqueous soluble inorganic anions, such as, fluoride, chloride, nitrite, nitrate, sulfate, and phosphate in starch hydrolysate products.

## 2. PRINCIPLE

An ion chromatography column with a sodium hydroxide mobile phase is used to separate anions based on their charge density and detected with a Pulsed Electrochemical Detector (PED) in the conductivity mode.

## 3. SCOPE

This method is applicable to all maltodextrin, corn syrup solids, and related products.

## 4. EQUIPMENT

The equipment listed below can be substituted with equivalent or higher quality equipment.

- 4.1 Dionex Series 4500 or DX500 Chromatography System containing an Eluant Degas Module (EDM), a Gradient Pump with a high-pressure GM-3 mixer (GPM), a microinjection valve with a 25 or 50  $\mu$ L loop, a Dionex Automated Sampler (DAS), a Pulsed Electrochemical Detector (PED) or CD20 conductivity detector, and an Advanced Computer Interface (ACI).
- 4.2 An IBM Pentium 75 MHZ compatible computer with a 200 Mb hard drive, Dionex AI-450 Chromatography software, Microsoft Windows Version 3.11 or greater, and a Okidata Microline 320 printer.
- 4.3 Dionex IonPac AS11 4 mm x 25 cm ion exchange column with an IonPac AG11 4 mm x 10 cm guard column.
- 4.4 Anion Trap Column (ATC-1).
- 4.5 Anion Self-Regenerating Suppressor (ASRS-II), 4 mm.
- 4.6 Self-Regenerating Suppressor (SRS) Controller.
- 4.7 Analytical balance capable of weighing  $200 \pm 0.0001$  g.
- 4.8 Lab-Line Mistral Multi-stirrer, 6 place, individually controlled.
- 4.9 Top loading balance capable of weighing at least  $200 \pm 0.01$  g.

000073

STANDARD ANALYTICAL METHOD Grain Processing Corporation Muscatine, Iowa 52761	Anion Screening Assay	Method No. C-56
Reviewed: 11/06/97 RIH <del>RIH</del> Revised: 11/06/97 Supersedes: New	Written by: RIH <del>RIH</del> Approved by: RMT <del>RIH</del> 11/7/97 Approval Auth: 46-00-01	Revision: 0 Page 2 of 9

4.10 Nylon disc filter, 25mm, 0.45 micron pore size

4.11 Autosampler vials and caps, 5 mL minimum volume

## 5. REAGENTS

### MAKE SURE ALL STORED REAGENTS AND SOLUTIONS CONTAIN A DATED LABEL AND NFPA CODE

- 5.1 Reverse Osmosis (RO)/Ion Exchanged Water: With a minimum resistivity of 200,000 ohms-cm (conductivity below 5.0  $\mu$ mho/cm) or water of similar quality.
- 5.2 Barnsted Water: Prepared as 18 Meg ohm by filtering RO water through a four cartridge system to remove organic and inorganic material. The Barnsted water is mobile phase solvent "B".
- 5.3 Sodium Hydroxide, NaOH, 50% w/w, low carbonate, Certified.
- 5.4 Sodium Hydroxide Solution, 25mM: Weigh  $4.00 \pm 0.01$  g of the 50% w/w NaOH into a tared two liter volumetric flask and dilute to volume with Barnsted water. Mix thoroughly. The 25mM NaOH is mobile phase solvent "A".
- 5.5 Sodium Hydroxide Solution, 1M: Weigh  $80.00 \pm 0.01$  g of 50% w/w NaOH into a tared one liter volumetric flask and dilute to volume with Barnsted water. Mix thoroughly. The 1M NaOH is used to regenerate the anion trap column.
- 5.6 Sodium Hydroxide Solution, 1mM: Weigh  $0.16 \pm 0.01$  g of 50% w/w NaOH into a tared two liter volumetric flask and dilute to volume with Barnsted water. Mix thoroughly.
- 5.6 Certified Five Anion Standard, Dionex Corporation.
- 5.7 Five Anion Working Standard: Prepare by pipetting 10 mL of the Certified Standard into a 100 mL volumetric flask with a volumetric pipet and diluting to volume with 1mM NaOH. Mix thoroughly and store both the Working Standard and the Certified Standard in a refrigerator at approximately 4 °C.

## 6. PROCEDURE

### 6.1 INSTRUMENT SETUP

- 6.1.a Fill each two liter solvent container with one of the mobile phase solvents, gently tighten the screw cap, and invert the container several times to mix the contents.



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- 6.1.b Attach each of the mobile phase solvent lines to the pump and set the Eluant Degas Module to sparge. Sparge the mobile phase for a minimum of 15 minutes prior to pressurizing the mobile phase containers and pumping mobile phase.
- 6.1.c While the mobile phase is degassing, setup the anion column hardware according to the instructions in 6.1.d - 6.1.i.
- 6.1.d Detach and remove any column hardware from previous analyses.
- 6.1.e Attach the Anion Trap Column in-line between the gradient pump and the microinjection valve.
- 6.1.f Place the mobile phase exit line from the microinjection valve into a beaker so that the system prior to the column can be flushed. **DO NOT ATTACH THIS LINE TO THE COLUMN UNTIL AFTER FLUSHING HAS BEEN COMPLETED.**
- 6.1.g At this time, the head of the AG-11 guard column should already be capped with a blocking nut and the guard column connected to the AS-11 separator column with approximately a 5 cm piece of Peek tubing. Make sure the flow direction arrows on the guard and separator columns are facing in the same direction as the mobile phase flow.
- 6.1.h The end of the separator column should be connected to the "eluent in" port of the Anion Self-Regenerating System (SRS). The flow direction will continue through to the "eluent out" port of the SRS and into the bottom of the Pulsed Electrochemical Detector (PED) cell.
- 6.1.i Connect the flow port on top of the PED cell into the "regen in" port of the SRS. A six to eight inch peek line should be connected to the "regen out" port and placed into a beaker. This line will later be connected to a waste line.
- 6.1.j **AT THIS TIME CONNECT THE ELECTRICAL LEAD FROM THE SRS CONTROLLER TO THE LEAD FROM THE ANION SRS. DO NOT TURN THE SRS CONTROLLER ON AT THIS TIME. MAKE SURE THE CURRENT SETTING ON THE SRS CONTROLLER IS ON "1".**

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<b>STANDARD ANALYTICAL METHOD</b> <b>Grain Processing Corporation</b> <b>Muscatine, Iowa 52761</b>	<b>Anion Screening Assay</b>	<b>Method No.</b> <b>C-56</b>
Reviewed: 11/06/97 RJH <del>11/11/97</del> Revised: 11/06/97 Supersedes: <u>New</u>	Written by: RJH <del>11/11/97</del> Approved by: RMT <del>11/11/97</del> RJH <del>11/11/97</del> Approval Auth: 46-00-01	Revision: 0 Page 4 of 9

6.1.k After the mobile phase sparging has been completed, pressurize the mobile phase container. Connect a 10-mL syringe with a luer lock fitting to the priming block, open the priming block valve and press the Start button on the Gradient Pump Module (GPM). Draw mobile phase into the syringe several times to ensure that all the air bubbles are removed from the incoming mobile phase line. Continue pumping mobile phase through the pump, microinjection valve, and into the beaker for at least five minutes.

6.1.l At this time, connect the mobile phase line from the microinjection valve to the head of the AG-11 guard column. **FIRST, TURN THE SRS CONTROLLER TO "ON". THEN, PRESS THE "START" BUTTON ON THE GRADIENT PUMP MODULE.**

6.1.m Now, observe the STATUS lights on the SRS Controller. When the system is Ready the green light will stay on. At this time the SRS is functioning properly and bubbles should be seen exiting the "regen out" line into the beaker.

6.1.n Allow the Gradient Pump Module's pressure to stabilize to a constant value and connect the waste line to the "regen out" line of the suppressor. Now, observe the pressure readings for five minutes to make sure that the pressure does not increase more than 40 psi. This is the back pressure for the anion SRS. If the pressure does increase more than 40 psi, stop the pump. If the pressure exceeds a 40 psi increase and operation of the system continues, the SRS internal membrane could be damaged and leaking from the SRS might be observed. If a blockage in the waste line is suspected, try flushing the line with mobile phase for several minutes to clear any salt obstruction and repeat instructions in 6.1.n.

6.1.o If the SRS back pressure does not exceed a 40 psi increase in pressure, continue pumping the mobile phase to equilibrate the system.

## 6.2 METHOD SETUP PARAMETERS

6.2.a Gradient Pump	
Flow rate	1.0 mL/min
Lower Pressure Limit	0 psi
Upper Pressure Limit	2000 psi
Run Time	40 min

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STANDARD ANALYTICAL METHOD Grain Processing Corporation Muscatine, Iowa 52761	Anion Screening Assay	Method No. C-56
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TIME	%A	%B	FLOW
0.00	5.0	95.0	1.0
2.40	5.0	95.0	1.0
4.00	5.0	95.0	1.0
25.00	80.0	20.0	1.0
25.10	5.0	95.0	1.0
40.00	5.0	95.0	1.0

### 6.2.b Conductivity Detector

Range	50 $\mu$ S
Temperature Compensation	1.7 $^{\circ}$ C
Output Voltage	1000 mV
Real Time Plot Scale	100 mV

### 6.2.c Integration and Calibration

Peak Width	9 seconds
Peak Threshold	2.0
Peak Area Reject	1000
Start Peak Detection	1.00 min
Double Bunching Factor	1.31 min
Double Peak Threshold	5.95 min
Force Baseline	5.95 min
Stop Peak Detection	25.00 min
Component Run Times	
Fluoride	2.60 min
Chloride	6.71 min
Nitrite	7.50 min
Nitrate	10.44 min
Sulfate	15.45 min
Phosphate	23.19 min

### 6.2.c Anion Suppressor Regenerating System

Membrane Current	300 mA
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## 6.3 STANDARD CURVE

6.3.a The Five Anion Certified Standard is used as the Primary standard. Six dilutions are prepared from the Primary standard covering a 100 fold dilution range listed in the table below.

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<b>STANDARD ANALYTICAL METHOD</b> <b>Grain Processing Corporation</b> <b>Muscatine, Iowa 52761</b>	<b>Anion Screening Assay</b>	<b>Method No.</b> <b>C-56</b>
Reviewed: 11/06/97 <del>RJH</del> Revised: 11/06/97 Supersedes: <u>New</u>	Written by: <del>RJH</del> Approved by: <del>RMT</del> 11/7/97 <del>RJH</del> 11/12/97 Approval Auth: 46-00-01	Revision: 0 Page 6 of 9

### **ANION STANDARD CURVE 100 FOLD DILUTION RANGE**

<b>ANIONS</b>	<b>LOW CONCENTRATION (PPM)</b>	<b>HIGH CONCENTRATION (PPM)</b>
FLUORIDE	0.204	20.4
CHLORIDE	0.297	29.7
NITRATE	0.99	9.9
PHOSPHATE	1.49	149.0
SULFATE	1.49	149.0

- 6.3.b All dilutions of the Secondary Standards were made with 1mM NaOH.
- 6.3.c Multiple injections should be made of each dilution; a minimum of three.
- 6.3.d Calculate the exact concentration of each anion in each of the Secondary Standards and enter these concentrations into the Calibration Section of the **Anion.met** method file.
- 6.3.e These standards can be run individually or automatically injected according to a **Schedule**. Setup the Schedule to run the standards under **Autocal**. Example: **AUTOCAL2R** designates the second standard level to be replaced; **AUTOCAL2A** designates the second standard level to be averaged. If the file designation is setup as **CALDATA** the software will add this data to the current method.
- 6.3.f Once the standard curve linear range has been established, calibration can be done daily with a single Working Standard.

### **6.4 SAMPLE PREPARATION OF SOLIDS**

- 6.4.a The amount of sample weighed will depend upon the concentration of the individual cations present. The following procedure will handle approximately 90% of the submitted samples.
- 6.4.b Accurately weigh approximately one gram of sample into a 100-mL volumetric flask. The more assays per sample completed the greater the accuracy of the data and the better the indication of homogeneity in the sample.

000078

<b>STANDARD ANALYTICAL METHOD</b> <b>Grain Processing Corporation</b> <b>Muscatine, Iowa 52761</b>	<b>Anion Screening Assay</b>	<b>Method No.</b> <b>C-56</b>
Reviewed: 11/06/97 RIH <del>RIH</del> Revised: 11/06/97 Supersedes: <u>New</u>	Written by: RIH <del>RIH</del> Approved by: RMT <del>RMT</del> 11/7/97 RIH <del>RIH</del> 11/12/97 Approval Auth: 46-00-01	Revision: 0 Page 7 of 9

6.4.c Fill the volumetric flask with approximately 50 mL of Barnsted water and dissolve the sample completely. Dilute the sample to volume with Barnstead water. Take approximately a 10 mL aliquot to filter through a 0.45 micron nylon disc filter. The filtrate can be put directly into the injection vials.

## 6.5 SAMPLE PREPARATION OF LIQUIDS

6.5.a The amount of the sample assayed will depend upon the concentration of the individual cations present. The following procedure will handle approximately 90% of submitted samples.

6.5.b Accurately pipet with an automatic pipettor one milliliter of sample into a 100 mL volumetric flask. Dilute to volume with Barnstead water and mix thoroughly.

6.5.c Filter approximately 10 mL of the diluted sample through a 0.45 micron nylon filter disc directly into the plastic injection vials.

## 7. CALCULATIONS

### 7.1 SOLID SAMPLES

$$\frac{A \times B}{C} = D$$

### 7.2 LIQUID SAMPLES

$$A \times B = D$$

A = Concentration of cation in  $\mu\text{g/mL}$  calculated from the standard curve.

B = Sample dilution in mL for solids; dilution fraction for liquids  
(example: 100 mL/1 mL).

C = Sample weight in grams.

D = Concentration of cation in the solid samples in  $\mu\text{g/g}$ ; and in  $\mu\text{g/mL}$  for liquids.

000079



<b>STANDARD ANALYTICAL METHOD</b> <b>Grain Processing Corporation</b> <b>Muscatine, Iowa 52761</b>	<b>Anion Screening Assay</b>	<b>Method No.</b> <b>C-56</b>
Reviewed: 11/06/97 RIH <del>V</del> Revised: 11/06/97 Supersedes: <u>New</u>	Written by: RIH <del>11/2/97</del> Approved by: RMT <del>11/2/97</del> RIH <del>11/2/97</del> Approval Auth: 46-00-01	Revision: 0 Page 8 of 9

## 8. VALIDATION

### 8.1 Accuracy

A blind sample was prepared containing five of the six calibrated standards and run a total six times. The calculated concentrations for each of the five anions were averaged to give the data summarized in the table below.

Anion	Mean Conc. ( $\mu\text{g/mL}$ )	$\pm$ SD ( $\mu\text{g/mL}$ )	% RSD	% Recovery
Fluoride	2.12	0.017	0.81	107.6
Chloride	3.18	0.059	1.85	105.3
Nitrate	10.32	0.053	0.515	102.2
Sulfate	17.43	0.110	0.632	116.2
Phosphate	14.59	0.204	1.40	97.9

### 8.2 Precision

The same sample used for the accuracy testing was also used to calculate the precision over six injections for each of the five anions calibrated. This data is summarized in the table that follows.

Anion	Mean Conc. ( $\mu\text{g/mL}$ )	$\pm$ SD ( $\mu\text{g/mL}$ )	% RSD	Precision at 95% Confidence Level
Fluoride	2.12	0.017	0.810	$\pm 1.62\%$
Chloride	3.18	0.059	1.85	$\pm 3.70\%$
Nitrate	10.32	0.053	0.515	$\pm 1.03\%$
Sulfate	17.43	0.110	0.632	$\pm 1.264\%$
Phosphate	14.59	0.204	1.40	$\pm 2.80\%$

000080

<b>STANDARD ANALYTICAL METHOD</b> Grain Processing Corporation Muscatine, Iowa 52761	Anion Screening Assay	Method No. C-56
Reviewed: 11/06/97 RIH <del>RIH</del> Revised: 11/06/97 Supersedes: <u>New</u>	Written by: RIH <del>RIH</del> Approved by: RMT <del>RMT</del> 11/17/97 RIH <del>RIH</del> 11/11/97 Approval Auth: 46-00-01	Revision: 0 Page 9 of 9

### 8.3 Minimum Detectable Quantities

The lowest concentration standard was diluted several times and injected to determine the minimum detectable quantity of each anion. Each anion response was evaluated for the smallest peak to be three times the baseline value. The injected minimum detectable quantities are listed in the summary table.

Anions	Minimum Detectable Quantity ( $\mu\text{g/mL}$ )
FLUORIDE	0.05
CHLORIDE	0.02
NITRATE	0.02
PHOSPHATE	0.05
SULFATE	0.05

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# ***SPI Polyols***

## STANDARD ANALYTICAL METHOD

METHOD NO. 9070

Page 1 of 7

### REDUCING SUGARS

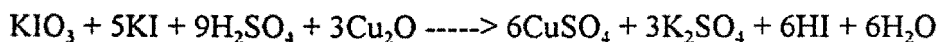
#### 1. SCOPE

This method is applicable to sorbitol, sorbitol solutions, and other polyol products. This procedure is the same as the NF method for the determination of Reducing Sugar in Noncrystallizing Sorbitol Solution, NF.

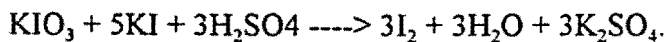
#### 2. PRINCIPLE

This method is applicable for the determination of sugars which reduce cupric ion in hot alkaline solution. Non-reducing or partially reducing sugars, such as sucrose, lactose, maltose, and the polysaccharides may be determined by this method after inversion to reducing sugars (see Total Sugar method #J-10-9087).

The reducing action of certain sugars on copper sulfate in boiling alkaline solution is the basis of the method. The copper ( $\text{Cu}^{++}$ ) is reduced to the insoluble (yellow to red) cuprous oxide ( $\text{Cu}_2\text{O}$ ). After cooling the solution, the  $\text{Cu}_2\text{O}$  is oxidized on acidifying the solution according to the following reaction:



The excess iodate and iodide react to release iodine as follows:



The liberated iodine is titrated with standard thiosulfate. The titration of the blank, less the titration of the sample, gives the milligrams (from a table) of  $\text{Cu}_2\text{O}$  reduced.

#### WARNING

Analytical methods involve potentially hazardous chemicals, analytical procedures and equipment, and in using these methods you assume all risk of personal injury and property damage. Information about the nature of these hazards and related safety precautions should be obtained before such chemicals, procedures and equipment are used. It is the responsibility of the user to take appropriate precautions.

The formation of  $\text{Cu}_2\text{O}$  is not a stoichiometric reaction. The amount of  $\text{Cu}_2\text{O}$  formed is not directly proportional to the amount of sugar present and the same amounts of different sugars give different amounts of  $\text{Cu}_2\text{O}$ . However, assuming a certain type of sugar present, it is possible to determine the amount from the  $\text{Cu}_2\text{O}$  reduced, using standardized tables. Thus, the difference between the blank titration and the sample titration of thiosulfate is converted to mg of glucose or invert sugar from the proper table.

### 3. REFERENCES

Bates, F. J. and Associates, "Polarimetry, Saccharimetry and the Sugars", circular of the National Bureau of Standards, C440, 1942.

Shaffer, P. A. and Hartman, A. F., J. Biol. Chem. 45, 377, 1921.

Somogyi, M., J. Biol. Chem. 70, 599, 1926.

NF 18 - 4th Supplement

### 4. SAFETY

Chemicals, reagents and samples used in this method may be considered hazardous. Refer to the material safety data sheets for information pertaining to the first aid, handling and disposal of the chemicals in this method.

### 5. EQUIPMENT

Dosimat, Brinkman model 725 or equivalent.

Glass beads, 4 mm, VWR Scientific #26396-563.

Condenser, Graham, Corning #2500, length of 400 mm, VWR Scientific #23136-047. Walter crucible holders, VWR Scientific #24065-000.

Flask, Erlenmeyer, Corning #4980, 300 ml capacity, VWR Scientific #29136-070.

Balance, analytical, capable of weighing 0.1 mg.

Timer, interval, Gar Lab, 60 minute in one minute intervals, VWR Scientific #62371-045.

Stirring bars, octagonal, 5/16" x 1/2", VWR Scientific #58948-116.

Water bath set at 20 C.

**000107**

## 6. CHEMICALS

Cupric sulfate-iodide solution (Alpha-Trol, Levittown, PA 1-800-319-2190) whose composition is as follows:

### Part 1:

Potassium citrate, monohydrate, ACS reagent grade	81 g
Potassium oxalate, monohydrate, ACS reagent grade	92 g
Potassium carbonate, NF granular monohydrate	83.6 g
(equivalent to 74 g of potassium carbonate, anhydrous)	
Dissolve in hot distilled water and dilute to 600 ml.	

### Part 2:

Cupric sulfate, pentahydrate, ACS reagent grade	25 g
Dissolve in hot water and dilute to 200 ml.	
Add to part 1 and mix for ½ hour.	

### Part 3:

Potassium iodate, ACS reagent grade	3.4 g
Potassium iodide, USP grade	50 g
Dissolve 0.4 g sodium hydroxide in hot distilled water. Add to the potassium iodate and potassium iodide and dilute to 200 ml. Add Part 3 to the mixture of Part 1 and Part 2 and stir at least 2 hours.	

Sulfuric Acid, 5.00 N +/- 0.02 N (VWR)

Sodium Thiosulfate, 0.1000 N +/- 0.005 N (VWR)

Starch Indicator, 1.0 % w/v with mercuric iodide (VWR)

## 7. ACCURACY AND REPEATABILITY

The accuracy within the laboratory for samples containing about 0.1 % reducing sugar is +/- 0.007% and the repeatability is 0.009%.

000108

**8. PROCEDURE**

1. Weigh into a 300 ml Erlenmeyer flask, to 2 decimal places, a sample weight using the calculation below (to contain between 50 to 125 mg of reducing sugar, if possible). For low weights, weigh to 4 decimal places. Use 30 g for finished sorbitol solution or crystalline sorbitol products (<0.3 % reducing sugar). For crystalline sorbitol slugs use 20 g to prevent gelling of sample. Prepare a blank in the same manner as the sample with the exception of the sample addition.

$$\text{gms of sample} = \frac{10}{\text{expected \% reducing sugar}} \quad (\text{Max sample is 30 g})$$

**NOTE:** For reducing sugar content over 16 %, weigh  $10.00 \pm 0.01$  g of sample into a 100 ml volumetric flask. Dilute with deionized water and mix. Pipet a suitable amount into the 300 ml Erlenmeyer flask.

$$\text{g of sample} = \frac{\text{g weighed into 100 ml vol flask} \times \text{ml pipetted}}{100}$$

2. Dilute to 50 ml with deionized water. Add 50.0 ml of the cupric sulfate-iodide solution using the dosimat. Add 3-4 glass beads.
3. Connect the flask to a water cooled reflux condenser with a Walter crucible holder. Heat on a hot plate adjusted to bring the solution to a boil within 3 minutes. Set a timer for 5 minutes and gently reflux the solution for 5 minutes.
4. After the 5 minute time interval, remove the flask and place it in a 20°C water bath for 15 to 25 minutes. DO NOT OVER COOL THE SAMPLE.
5. Add a stir bar. Using the dosimat, add slowly 25 ml of 5 N sulfuric acid solution, stirring during and between the additions. Watch for foaming.

**NOTE:** DO NOT PLACE HAND OVER THE TOP OF THE FLASK TO STOP FOAMING. Adding the acid slowly while swirling will decrease the amount of foam.

6. Using a dosimat, titrate the liberated iodine with 0.1 N sodium thiosulfate titrant to a pale green color, then add 1 ml of starch indicator solution. Continue the titration to a pale green-blue endpoint.

000109

**9. CALCULATIONS**

Thio blank - Sample titration = Titration difference.

From the appropriate attached table [Table A (Invert) or Table B (Glucose)], find the mg of sugar equivalent to the titration difference. Then calculate the % reducing sugar in the sample.

$$\text{Reducing Sugar, \% (as is)} = \frac{\text{mg of sugar from chart}}{10 \times \text{g of sample}}$$

$$\text{Reducing Sugar, \% (at 70\% solids)} = \frac{\text{mg of sugar from chart} \times 70}{10 \times \text{g of sample} \times \% \text{ solids}}$$

$$\text{Reducing Sugar, \% (dry basis)} = \frac{\text{mg of sugar from chart} \times 100}{10 \times \text{g of sample} \times \% \text{ solids}}$$

$$\text{Reducing Sugar, \% (at 70\% solids = 10 x g of sample x \% solids x 80} \\ \text{correcting for 20 \% mannitol)}$$

**10. DOCUMENT CHANGE RECORD**

<u>Rev. No.</u>	<u>Eff. Date</u>	<u>Change</u>
0	11/1/77	None - New Work Instruction

**11. APPENDIX**

Appendix A - mg sugar table for PAI products (Invert )

Appendix B - mg sugar table for SORBO<sup>R</sup> products (Glucose)

**000110**

Appendix A, mg of Sugar Table for PAI Products\* (Invert)

ml diff	.00	.10	.20	.30	.40	.50	.60	.70	.80	.90
0	-	-	1.66	2.14	2.44	2.82	3.10	3.40	3.68	3.96
1	4.14	4.42	4.80	5.08	5.36	5.76	6.06	6.34	6.62	6.90
2	7.18	7.46	7.74	8.12	8.44	8.70	9.10	9.38	9.66	9.94
3	10.22	10.50	10.78	11.16	11.46	11.74	12.02	12.32	12.60	12.88
4	13.16	13.44	13.72	14.00	14.30	14.58	14.86	15.16	15.44	15.72
5	16.10	16.38	16.66	16.94	17.22	17.52	17.80	18.00	18.38	18.66
6	18.94	19.32	19.60	19.88	20.18	20.46	20.74	20.96	21.12	21.40
7	21.68	21.96	22.24	22.52	22.82	23.10	23.38	23.68	23.96	24.24
8	24.52	24.70	24.98	25.26	25.46	25.74	26.02	26.32	26.60	26.88
9	27.16	27.44	27.62	27.90	28.20	28.48	28.76	29.06	29.34	29.53
10	29.70	29.99	30.27	30.44	30.76	31.03	31.21	31.60	31.89	32.17
11	32.54	32.84	33.11	33.38	33.70	34.07	34.35	34.74	35.04	35.31
12	35.58	35.88	36.15	36.43	36.72	37.02	37.29	37.58	37.88	38.25
13	38.53	38.90	39.19	39.57	39.86	40.16	40.43	40.73	41.00	41.29
14	41.57	41.84	42.14	42.41	42.70	43.00	43.27	43.57	43.84	44.14
15	44.41	44.68	44.98	45.25	45.55	45.82	46.12	46.41	46.68	46.98
16	47.25	47.53	47.90	48.19	48.49	48.76	49.06	49.35	49.63	50.00
17	50.29	50.57	50.84	51.14	51.43	51.71	52.00	52.29	52.57	52.84
18	53.14	53.41	53.68	53.98	54.27	54.55	54.82	55.14	55.41	55.63
19	55.98	56.35	56.73	57.00	57.32	57.69	57.96	58.28	58.65	58.93
20	59.20	59.49	59.87	60.14	60.44	60.73	61.01	61.30	61.59	61.87
21	62.14	62.44	62.81	63.08	63.40	63.77	63.95	64.44	64.74	65.01
22	65.28	65.58	65.85	66.13	66.40	66.74	66.99	67.26	67.58	67.95
23	68.23	68.60	68.89	69.17	69.44	69.76	70.03	70.33	70.60	70.89
24	71.17	71.44	71.74	72.01	72.28	72.60	72.87	73.17	73.34	73.64
25	73.91	74.08	74.38	74.65	74.83	75.02	75.33	75.62	75.79	76.09

\*For PAI, SORBO<sup>R</sup> I, SUTRO<sup>R</sup> products.

000111



## METHOD NO. 9070

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Appendix B, mg of Sugar Table for SORBO<sup>®</sup> Products\* (Glucose)

ml diff	.00	.10	.20	.30	.40	.50	.60	.70	.80	.90
0	-	-	1.26	1.74	2.04	2.42	2.90	3.20	3.48	3.76
1	4.04	4.32	4.60	4.88	5.16	5.46	5.86	6.14	6.42	6.70
2	6.98	7.26	7.54	7.92	8.24	8.50	8.80	9.08	9.36	9.54
3	9.72	10.00	10.28	10.56	10.86	11.14	11.42	11.72	12.00	12.28
4	12.56	12.84	13.12	13.40	13.70	13.98	14.26	14.56	14.84	15.12
5	15.30	15.58	15.86	16.14	16.42	16.72	17.00	17.20	17.48	17.76
6	18.04	18.32	18.60	18.88	19.18	19.46	19.74	20.04	20.32	20.60
7	20.88	21.16	21.44	21.72	22.02	22.30	22.58	22.88	23.16	23.44
8	23.72	24.00	24.28	24.56	24.86	25.14	25.42	25.72	26.00	26.28
9	26.56	26.84	27.22	27.50	27.80	28.08	28.36	28.66	28.94	29.33
10	29.60	29.89	30.17	30.44	30.76	31.03	31.41	31.70	31.99	32.27
11	32.54	32.84	33.11	33.48	33.80	34.07	34.35	34.64	34.94	35.21
12	35.58	35.88	36.15	36.43	36.72	37.02	37.29	37.68	37.88	38.25
13	38.53	38.80	39.09	39.37	39.66	39.96	40.23	40.53	40.80	41.09
14	41.37	41.64	41.94	42.21	42.50	42.80	43.07	43.37	43.74	44.04
15	44.31	44.58	44.88	45.15	45.45	45.82	46.12	46.41	46.68	46.98
16	47.25	47.53	47.90	48.19	48.49	48.76	49.06	49.35	49.63	50.00
17	50.29	50.57	50.84	51.14	51.43	51.71	52.00	52.39	52.67	52.94
18	53.24	53.51	53.78	54.08	54.47	54.75	55.02	55.34	55.61	55.88
19	56.18	56.55	56.83	57.10	57.42	57.69	57.96	58.28	58.65	58.93
20	59.20	59.49	59.87	60.14	60.44	60.73	61.01	61.30	61.59	61.87
21	62.14	62.44	62.81	63.08	63.40	63.67	63.95	64.24	64.54	64.91
22	65.18	65.48	65.75	66.03	66.30	66.64	66.99	67.26	67.58	67.85
23	68.13	68.40	68.69	69.07	69.34	69.66	69.93	70.23	70.50	70.79
24	71.17	71.44	71.74	72.01	72.28	72.60	72.87	73.27	73.54	73.84
25	74.11	74.38	74.68	74.95	75.33	75.62	75.93	76.32	76.59	76.89

For A-641, A-625, AHS-625, A-810, SORBO<sup>®</sup>, PAS-J3, Crystalline Sorbitol, ARLEX<sup>®</sup>, Sorbitol Special<sup>™</sup> products.

000112

# SPI Polyols

## STANDARD ANALYTICAL METHOD

METHOD NO. 9859

Page 1 of 9

### WATER, %

#### 1. SCOPE

This method is applicable to the determination of water in polyol products. The samples must be soluble or dispersible in the reagent.

This method cannot be used, except with modifications, with materials that react with the reagent. Examples are active aldehydes and ketones, strong amines, amino alcohols, hydrazine salts, propylene oxide, thioureas, peracids, diacyl peroxides, ethylene oxide, and sucrose.

#### 2. PRINCIPLE

The Karl Fisher method is based upon titrimetric determination of the reaction of water with the Karl Fisher reagent. The reagent reacts with the water in the sample to give an apparent resistance change due to the depolarization effect on the electrode. The reagent is standardized against water, as primary standard. This method is consistent with Method <921> Method 1a in USP 23.

#### 3. REFERENCES

Applications Manual, AquaStar V1-B Volumetric Titrator, EM Science.

USP 23 / NF 18

#### **WARNING**

Analytical methods involve potentially hazardous chemicals, analytical procedures and equipment, and in using these methods you assume all risk of personal injury and property damage. Information about the nature of these hazards and related safety precautions should be obtained before such chemicals, procedures and equipment are used. It is the responsibility of the user to take appropriate precautions.

#### 4. SAFETY

Chemicals, reagents and samples used in this method may be considered hazardous. Refer to the material safety data sheets for information pertaining to the first aid, handling and disposal of the chemicals in this method.

#### 5. EQUIPMENT

AQUASTAR V1B Volumetric Titrator, EM Science, EM-AX1695B-1.

AQUASTAR Printer, EM Science, EM-A3210171-1.

Balance, Mettler, P83001 (0.1g precision, 3100 g capacity, or equivalent).

Balance, Mettler, AC100 (0.05 mg precision, 1008 g capacity, or equivalent).

Beaker, 50 ml , to weigh samples for Aquatrator, or small paper souffle cups can be used.

Bottle, dropping, Kimax, with interchangeable pipette and rubber bulb, capacity 30 ml.

Flask, Erlenmeyer, Pyrex brand glass, capacity 50 ml, Corning Catalog No. 4980.

Pipette, dropping, disposable, LDPE 68 mm size

Replacement Container, Pyrex Jar, 1 Quart, Emulsifying Mill, Blender, Waring No. 700-510.

Souffle Cups.

Syringes, disposable, 1 cc and 5 cc.

TPT-71 twin platinum electrode, AQUASTAR, A7108061.

Waring Blender, laboratory model, Waring Products Corp., Winsted, Conn., Model 700-S.

## 6. CHEMICALS

Chromerge, such as Fisher Scientific, Cat No C57712.

Drierite, such as Fisher Scientific, Cat. No. 07-577.

KARL FISHER REAGENT, pyridine free, such as EM Science AQUASTAR Comp. 5, Cat. No. AX1698A-7.

Methanol, anhydrous, such as Baker Analyzed HPLC Reagent, Cat. No. 9093-33.

Sulfuric acid reagent grade.

## 7. REAGENTS

### Chromerge Cleaning Solution

### **WEAR GLOVES, FACE SHIELD & PERFORM IN HOOD**

1. Carefully add a 25 ml vial of Chromerge to new 2.5 L bottle of concentrated Sulfuric Acid. Mix well and transfer to a 1 gallon amber bottle. Store in hood when not in use.

## 8. GENERAL INSTRUCTIONS

1. The temperature of the area in which the apparatus is used should be between 60°F and 85°F. The relative humidity should not exceed 70 %. The temperature and humidity will be monitored and recorded once per week. If unusual circumstances exist, the temperature and humidity will be recorded as appropriate.
2. The Karl Fisher apparatus should be placed in a relatively draft free area.
3. The level of the Karl Fisher reagent in the bottle should be checked to determine if it is sufficient to last through the work day. If not, a fresh bottle should be installed and standardized. Any reagent used should have been stabilized at room temperature. (Standing in the laboratory for 8 hours is usually sufficient).
4. Check any drying tubes on the Karl Fisher apparatus and change the Drierite when necessary.
5. To maintain adequate end-point sensitivity, drain reaction vessel frequently. This draining practice is for cleaning purposes and the frequency is determined by the types of samples being analyzed.

6. If the electrode tips become coated over titration can result. To correct this, the electrode tips should be wiped gently with a tissue and immersed in hot Chromerge cleaning solution for 10-15 minutes.
7. All connections and stopcocks should be routinely inspected, and tightened if required, to prevent leakage.
8. Keep system closed as much as possible. Do not open reaction vessel except to introduce sample, cleaning or maintenance.
9. Before standardization and/or running the analytical test sample, the reaction vessel should be drained, rinsed thoroughly with dry methanol, closely inspected for cleanliness, taken apart and cleaned if necessary. Fill the vessel with dry methanol. Press blank to blank out the water in methanol. (Solution must cover tip of electrode)
10. The Karl Fisher reagent in the reaction vessel must always be at room temperature except where sample solubility requires heating.
11. During titration, the solution in the reaction vessel is stirred at such a rate to provide rapid mixing, but not so fast as to cause the solution to be splashed on the walls or cover of the vessel above the solution.

#### Instrument Settings

Minimum Rate	-	5
End Point Time	-	20 sec.
Prop Band	-	4
Current Adjust	-	10.
Stirrer Switch	-	ON
End Point	-	1
Auto Balance Switch	-	OFF

Basic Operating Parameters

The basic operating parameters and keyboard sequence to set them are as follows:

INTERVAL	30	ENTER	(end point hold time)
OPT	1	ENTER	(burette speed)
	1	ENTER	(Mode 1-20 ml titrant/min.)
OPT	2	ENTER	(Indication and alarm of remaining reagent)
	9999	ENTER	(initial mls)
OPT	3	ENTER	(maximum amount of titration)
	100	ENTER	(100 mls)
OPT	4	CE, ENTER	(titration curve will not print)
OPT	5	ENTER	(titration timer)
	0	ENTER	(timer not active)
OPT	6	ENTER	(start titration timer)
	0	ENTER	(timer not active)
OPT	7	ENTER	(blank titration)
	0	ENTER	(no blank titration)
OPT	8	ENTER	(automatic background correction)
	0	ENTER	(no background correction)

9. STANDARDIZATION

1. Karl Fisher reagent in high water instrument reaction vessel must be at room temperature and 'just' neutral or blanked out. Adjust as necessary by pressing the 'Blank' button. The low water instrument reaction vessel is heated to aid in sample dissolution.
2. Press 'CAL MODE' key then 4 then enter.
3. Push the 'SAMPLE' key (This suspends titration until TITRATION is pushed).
4. From a 1 cc disposable syringe add approximately 0.10 gm of deionized water into the reaction vessel.
5. Immediately proceed with titration by pushing the 'TITRATION' key. The amount of water titrated should consume 15-20 mls of Karl Fisher reagent.
6. Weigh the syringe by difference to nearest 0.0001 gm. Enter sample weight into instrument by pressing 'SAMPLE SIZE', the weight in grams and the enter key. The titration volume and the factor are displayed on the printout.

7. Repeat steps 3 thru 6 until 3 consecutive factor determinations agree within 0.010.

$$\text{Factor Determination} = \frac{\text{gm of water} \times 1000}{\text{mls titration}}$$

The factor is the average of the 3 consecutive determinations that agree within 0.010 and is calculated to the third decimal place.

8. Enter the factor into the titrator using the following keyboard sequence:
- Push the 'FACTOR' key.
  - Enter the factor.
  - Push the 'ENTER' key.
  - Enter the date (use . to separate the month, day, and year).
  - Push the 'ENTER' key.
9. Press 'CAL MODE' key then 0 (zero) then enter.
10. Analyze either the high or low water Assurance Test Sample.
11. Analyze the High Water Assurance Test Sample for % water. Weigh sample (approximately 0.4 gm, 7 drops, weighed to nearest 0.0001 gm) by difference from a disposable pipette. Use the method as outlined in steps 3 - 6 above. When the titration is complete the % water will be printed out.
12. Analyze the Low Water Assurance Test Sample for % water. Weigh sample (approximately 3-5 grams) by difference from a soufflé cup. Use the method as outlined in steps 3 - 6 above. When the titration is complete the % water will be printed out.

Duplicate % water results, within the established range and that check each other within 0.4% for the high water ATS or 0.06% for the low water ATS, should be obtained to assure the factor is correct.



**10. ACCURACY AND REPEATABILITY**

The accuracy within the laboratory for the low water ATS (approximately 0.4 % water) is  $\pm 0.05\%$  and the repeatability is not more than 0.06%. The accuracy within the laboratory for the high water ATS (approximately 30 % water) is  $\pm 0.29\%$  and the repeatability is not more than 0.36%. The Low Water Instrument is used for determinations up to 5%. The High Water Instrument is used for all other determinations.

**11. PROCEDURE**

1. For crystalline sorbitol samples refer to Appendix 1 for sample preparation.
2. Blank out the solution in the reaction vessel. Karl Fisher reagent in reaction vessel must be at room temperature for High Water Instrument (Samples > 5% water). For the Low Water Instrument (Samples < 5% water) the Karl Fisher reagent in the reaction vessel must be heated to not greater than 70 °C to dissolve samples. Re-blank solution before starting analysis as necessary.
3. Push the 'SAMPLE' key (This suspends titration until the 'TITRATION' key is pushed).
4. Choose an appropriate amount of sample and weigh into the sample vessel by difference according to the following table:

<u>Expected % Water</u>	<u>Sample Weight g **</u>	<u>Weigh to</u>
0.5 or less *	19.2	2 decimal places
1 *	9.6	'
2 *	4.8	3 decimal places
3	3.2	'
5	1.9	'
10	1.0	4 decimal places
15	0.6	'
20	0.5	'
30	0.32	'
40	0.24	'
50	0.19	'
60-100	0.16-0.10	'

\* For molten, use 4 grams. For crystalline sorbitol and sorbitol slugs, see Appendix 1.

\*\* These sample weights are set such that a titration of about 17.5 ml is achieved with a KF reagent that has a factor of 5.5.

5. Immediately proceed with titration by pushing the 'TITRATION' key.
6. Enter sample weight into instrument by pressing the 'SAMPLE SIZE' key, and then entering the weight in grams followed by the 'ENTER' key. The result in percent water will appear on the instrument printout.
7. Repeat steps 2 thru 6 until duplicate determinations agree within 0.20% for samples run on the High Water Instrument. All crystalline sorbitol samples run on the Low Water Instrument are run as a single determination.

## 12. CALCULATIONS

$$\% \text{ Water} = \frac{\text{Sample Titration} \times \text{Factor} \times 0.1}{\text{Grams Sample}}$$

## 13. DOCUMENT CHANGE RECORD

<u>Rev. No.</u>	<u>Eff. Date</u>	<u>Change</u>
0	6/16/97	This method was updated and combined from ICI SAM 9859 and 9859D. SAM's 9859A-C were omitted from this revision because they pertain to surfactant products. Appendix 1 reflects the combination of SAM's 9859 and 9859D.
1	11/1/97	Specified temperature and humidity control limits. Changed factor to three <u>consecutive</u> determinations must be within 0.010. Changed sample table to reflect the current KF titer.

**14. APPENDIX 1****KARL FISCHER METHOD FOR DETERMINATION OF WATER IN  
CRYSTALLINE SORBITOL****1. Crystalline Sorbitol Slugs**

- A. Grind the contents of a 250-ml beaker, 3/4 full of slugs, in the Waring Blender at full speed for one minute, using an automatic timer.
  - 1. Caution: Be sure that the cap of the Waring Blender jar is securely in place prior to grinding.
  - 2. Make sure that the Waring Blender glass container is clean, dry and at room temperature. Wash with water, wipe dry, and place in drying oven for 2-3 minutes. Do not overheat the glass. The container must be cool when sample is ground.
- B. Filter the sample through a #40 screen to get rid of large pieces.
- C. Transfer a portion of the filtered sample into a paper cup. Samples must be analyzed immediately after grinding and must be weighed quickly to avoid moisture pick-up.
- D. Continue by following instructions as given in the Karl Fischer Water Method No. 9859 Procedure Section beginning with STEP 2. Use a 3-4 gram sample size. All samples should be dissolved completely in the Karl Fischer reagent before titration is initiated. All crystalline sorbitol samples are run as a single determination.

**2. Crystalline Sorbitol Powder**

- A. Transfer a portion of the ground sample into a paper cup, avoiding large pieces. Samples must weighed quickly to avoid moisture pick-up.
- B. Continue by following instructions as given in the Karl Fischer Water Method No. 9859 Procedure Section beginning with STEP 2. Use a 3-4 gram sample size. All samples should be dissolved completely in the Karl Fischer reagent before titration is initiated. All crystalline sorbitol samples are run as a single determination.

**000121**

METHOD NO. 2740

Page 1 of 2

**RESIDUE ON IGNITION (SULFATED ASH)****Scope**

This method is applicable for the determination of "residue on ignition" of polyols. It is the official USP test.

**Reference**

United States Pharmacopeia XXII Revision, 1990.

**Principle**

The sample is charred in crucible and treated with sulfuric acid to convert the inorganic residue to sulfated salts.

**Apparatus**

Crucible, Coors, porcelain, high form, 40 ml capacity, Fisher #07-965F.

Tongs, stainless steel, 23 cm length, Fisher #15-186.

Muffle furnace, temperatures of 200 to 1093°C, Fisher #10-552.

Bunsen burner, with adjustable flame height, Fisher #02-962P (for propane).

Hot plate, heats to 243°C, Fisher #11-496-3.

Stand, cast-iron, rectangular, base size 4" x 6", Fisher #14-670A.

**WARNING**  
Stand, cast-iron, rectangular, base size 4" x 6", Fisher #14-670A.

Analytical methods involve potentially hazardous chemicals, analytical procedures and equipment, and in using these methods you assume all risk of personal injury and property damage. Information about the nature of these hazards and related safety precautions should be obtained before such chemicals, procedures and equipment are used. It is the responsibility of the user to take appropriate precautions.

Ring, cast-iron, 3" o.d., Fisher #14-050B.

Balance, analytical, capable of weighing accurately to  $\pm 0.1$  mg.

Graduated medicine dropper, 2 ml capacity, Fisher #13-703B.

#### Reagents

Sulfuric acid, reagent grade, ACS, Fisher #ΔA-300.

#### Procedure

1. Weigh, to 0.0001 gr, a clean porcelain crucible.
2. Weigh about 2 gr (to 0.1 mg) of sample into the crucible.
3. Burn the sample to a black char by using a hot plate and/or a Bunsen burner.

Note: This procedure, Steps 3 through 6 should be performed under a hood.

4. Cool the sample.
5. Moisten the residue with 1 ml of sulfuric acid using a graduated medicine dropper.
6. Heat gently until white fumes no longer are evolved and then place in muffle furnace at  $800 \pm 25^\circ\text{C}$  until the carbon is completely consumed.

NOTE: Conduct the ignition in a place protected from air currents, and use as low a temperature as possible to effect the combustion of carbon.

7. When the carbon has completely disappeared, cool the crucible in a desiccator.
8. Reweigh crucible to 0.0001 gr.

#### Calculation

$$\frac{\% \text{ Sulfated ash}}{(\text{Residue on ignition})} = \frac{\text{g of residue} \times 100}{\text{g of sample}}$$

NOTE: If the amount of residue exceeds 0.1% ignite to constant weight, and again calculate the percentage of residue.

METHOD NO. 5181

Page 1 OF 3

**HEAVY METALS (USP) IN POLYOLS**Scope and Status

This is the USP method for detecting any metallic impurities in polyols that are colored by hydrogen sulfide under the conditions of the test (Ag, As, Bi, Cu, Hg, Pb, Sb, Sn). It will detect these impurities in the treated samples by comparing the color of the sample with the color produced by a standard solution of lead to a limit of 10 ppm by weight.

Reference

Pharmacopeia of the United States, XVII Revision, 1965, pp. 661, 662, 876, 877.

Apparatus

Nessler tubes, 50 ml, matched color.

Flasks, volumetric, 100 ml, 1000 ml.

Pipettes, volumetric, 2 ml, 10 ml.

Graduated cylinder, 10 ml.

Beaker, 50 ml.

Stirring rod, glass, with a loop at one end.

**WARNING**

Analytical methods involve potentially hazardous chemicals, analytical procedures and equipment, and in using these methods you assume all risk of personal injury and property damage. Information about the nature of these hazards and related safety precautions should be obtained before such chemicals, procedures and equipment are used. It is the responsibility of the user to take appropriate precautions.

Reagents

Nitric acid, reagent grade, USP XVII, 1965, p. 1010.

Lead nitrate stock solution. Dissolve 159.8 mg of lead nitrate in 100 ml of water to which has been added 1 ml of nitric acid, then dilute with water to 1000 ml, 1 ml = 0.0001 g Pb. Prepare and store this solution in glass containers free from soluble lead salts.

Standard lead solution. Measure 10 ml of lead nitrate stock solution with a volumetric pipette and transfer it to a 100 ml volumetric flask. Dilute to the mark with distilled water, 1 ml = 0.00001 g Pb. Prepare this solution fresh on the day of analysis.

Acetic acid, diluted (1N), USP XVII, 1965, p. 934. Dilute 60.0 ml of glacial acetic acid with sufficient water to make 1000 ml.

Hydrogen sulfide T.S., USP XVII, 1965, p. 1076. Prepare a saturated solution of hydrogen sulfide gas into cold water. Store in small, dark, amber-colored bottles, filled nearly to the top. It is unsuitable for use unless it possesses a strong odor of hydrogen sulfide and unless it produces a copious precipitate of sulfur when added to an equal volume of ferric chloride T.S. Store in a cold, dark place.

Ferric chloride T.S. (1N), USP XVII, 1965, p. 1075. Dissolve 9 g of ferric chloride in sufficient water to make 100 ml of solution.

pH indicator paper, pH 3-4.

Ammonia T.S., USP XVII, 1965, p. 1072. It contains between 9.5 and 10.5% of  $\text{NH}_3$ . Prepare by diluting 400 ml of strong ammonia water (p. 940) with sufficient distilled water to make 1000 ml. The heavy metals limit of the ammonia T.S. used in this test is 2 ppm.

Procedure

1. Weigh 2.0 g of sorbitol solution into a 50 ml beaker.
2. Add 2 ml of diluted acetic acid.
3. Wash the solution into a 50 ml Nessler tube with distilled water to a total volume of 25 ml and mix.
4. Into a matching 50 ml Nessler tube, measure 2 ml of standard lead solution with a volumetric pipette.

000125

5. Dilute this solution to 25 ml with distilled water and mix.
6. Adjust the pH of the sample and the standard lead solution with dilute acetic acid or ammonia T.S. to between 3 and 4 (using pH indicator paper).
7. Dilute each solution to 40 ml and mix.
8. To each tube, add 10 ml of freshly prepared hydrogen sulfide T.S. Mix with a stirring rod containing a loop at the lower end.
9. Allow to stand for 5 minutes, then view downward over a white surface.
10. If the color of the sorbitol sample is not darker than the color of the standard lead solution (10 ppm), report "passes test."



METHOD NO. 3061

Page 1 of 3

**DETERMINATION OF CHLORIDE IN POLYOLS**Scope and Status

This is the USP method used to detect the presence of chlorides in polyols.

Reference

Pharmacopeia of the United States, XXII, 1990.

Principle

An acidified sample of polyol is treated with silver nitrate T.S. which precipitates chlorides, if they are present. The turbidity produced in the sample is compared with the turbidity of a standard solution of hydrochloric acid containing the same quantity of reagents as the sample.

Apparatus

Nessler tubes, 50 ml.

Nessler tube rack.

Beaker, 50 ml.

**WARNING****000127**

Analytical methods involve potentially hazardous chemicals, analytical procedures and equipment, and in using these methods you assume all risk of personal injury and property damage. Information about the nature of these hazards and related safety precautions should be obtained before such chemicals, procedures and equipment are used. It is the responsibility of the user to take appropriate precautions.

Apparatus (Cont'd.)

Pipette, 1 ml, Mohr with 0.10-ml graduations.

Pipette, 1 ml.

Flask, volumetric, 100 ml.

Reagents

Nitric acid, reagent grade, USP.XXII

Silver nitrate T.S., 0.1N, USP. XXII

Dissolve about 17.5 gr of silver nitrate in 1000 ml of distilled water.

Hydrochloric acid, 0.02N. Accurately measure 20 ml of standardized 0.100N hydrochloric acid into a 100-ml volumetric flask. Dilute to the mark with distilled water.

Procedure

1. Weigh 1.5 g of sample into a 50-ml beaker and dissolve with 30 to 40 ml of distilled water. Pour into a 50-ml Nessler tube.

Note: Weigh 3.0 g sample to test for compliance with Atlas specification of 25 ppm maximum chlorides.

2. Neutralize the sample solution to litmus with nitric acid.

Note: If the sample is not clear at this point, it should be filtered through filter paper that gives a negative test for chlorides and sulfates.

3. Into a second matching Nessler tube, pour enough chloride-free distilled water to equal the volume of liquid in the sample tube.
4. Add 0.1 ml of 0.02N hydrochloric acid to the Nessler tube containing the water and mix.

5. To each tube add:  
  
1 ml nitric acid and mix.  
1 ml of silver nitrate T.S.  
Enough distilled water to make 50 ml. Mix well.
6. Allow to stand for 5 minutes protected from direct sunlight, then compare the turbidity of the sample with the turbidity of the standard.
7. If the turbidity of the sample is not greater than the turbidity of the standard (50 ppm) report "passes test." If the turbidity of the sample is greater than the turbidity of the standard, report "does not pass test."

Note: If the turbidity of the 3.0-g sample is less than the standard, report "<25 ppm chloride".

METHOD NO. 9220

Page 1 of 4

**SULFATES, PPM****1. SCOPE**

This USP method is applicable for the determination of the sulfate content (ppm) of sorbitol and sorbitol solutions.

**2. PRINCIPLE**

An acidified sample of sorbitol is treated with barium chloride which will precipitate sulfates, if they are present. The turbidity of the solution is compared with the turbidity of an equal volume of deionized water containing a standard quantity of sulfate which also has been treated with barium chloride.

**3. REFERENCES**

USP XXIII / NF XVIII

**4. SAFETY**

Chemicals, reagents and samples used in this method may be considered hazardous. Refer to the material safety data sheets for information pertaining to the first aid, handling and disposal of the chemicals in this method.

**5. EQUIPMENT**

Balance, analytical, capable of weighing 0.1 mg.

Beaker, 50 ml capacity, VWR Scientific #13910-143.

Color comparison tubes, Nessler, APHA specification, tall form, matched, 50 ml capacity, VWR Scientific #66176-507.

**WARNING**

Analytical methods involve potentially hazardous chemicals, analytical procedures and equipment, and in using these methods you assume all risk of personal injury and property damage. Information about the nature of these hazards and related safety precautions should be obtained before such chemicals, procedures and equipment are used. It is the responsibility of the user to take appropriate precautions.

**000130**

Filter paper, sulfate free, prepleated, 15 cm, VWR Scientific #28420-046.

Flask, volumetric, Kimble #28014G, with PTFE stopper, 100 ml capacity, VWR Scientific #29619-676.

Flask, volumetric, Kimble #28014F, with PTFE stopper, 500 ml capacity, VWR Scientific #29619-679.

Funnel, filling, Nalgene #4252, 100 mm top diameter, VWR Scientific #30255-066.

Graduated cylinder, 500 ml capacity, VWR Scientific # 24710-180.

Graduated cylinder, 100 ml capacity, VWR Scientific # 24710-124.

Litmus paper, red, VWR Scientific #60795-007.

Pipet, Mohr, 1 ml capacity, in 0.01 increments (VWR Scientific #52969-066)

Pipet, volumetric, Corning #7100A, Cortex, Class A, 1 ml capacity, VWR Scientific #53044-037.

Pipet, volumetric, Corning #7100A, Cortex, Class A, 3 ml capacity, VWR Scientific #53044-070.

Tube support, capacity to hold 12 (50 ml) Nessler tubes, VWR Scientific #66176-722.

## 6. CHEMICALS

Barium chloride, dihydrate, certified, ACS, VWR Scientific #EMBX0060-1.

Hydrochloric acid, 36.5-38 %, certified, ACS, Safe-Cote bottle, VWR Scientific #JT9535-33.

Sulfuric acid, 0.1 N, certified, ACS, VWR Scientific #VW-3230-1.

## 7. REAGENTS

### 12 % Barium Chloride Solution

1. Into a 100 ml volumetric flask, weigh  $12.0 \pm 0.1$  g of barium chloride. Dissolve in a few ml of deionized water.
2. Dilute to the mark with deionized water. Mix well.

3 N Hydrochloric Acid Solution

**WEAR SAFETY GOGGLES, APRON & LATEX GLOVES WHILE HANDLING CONCENTRATED ACID.**

1. Into a 500 ml volumetric flask add approximately 250 ml of deionized water.
2. Under a hood, carefully add 118 ml of hydrochloric acid into the volumetric flask containing 250 ml of deionized water.
3. Dilute to the mark with deionized water. Mix well.

0.020 N Sulfuric Acid Solution

**WEAR SAFETY GOGGLES, APRON & LATEX GLOVES WHILE HANDLING ACID.**

Into a 100 ml volumetric flask add approximately 50 ml of deionized water. Under a hood, carefully add 20 ml of 0.10 N sulfuric acid. Dilute to the mark with deionized water. Mix well.

8. PROCEDURE

1. Weigh  $2.0 \pm 0.01$  g of sample into a 100 ml beaker.
2. Add approximately 30 ml of deionized water. Heat to boiling to dissolve the sample with stirring.
3. Neutralize the solution to litmus with 3N hydrochloric acid.
4. If cloudy, filter the solution through filter paper.
5. Wash the contents of the beaker into a 50 ml Nessler tube.
6. Into a separate Nessler tube, add 0.10 ml of 0.02 N sulfuric acid. Add as much deionized water to equal the volume in the sample tube. This is the standard.
7. To both beakers, add 1 ml of 3 N hydrochloric acid solution and 3 ml of 12 % barium chloride solution. Add sufficient deionized water to bring each tube to the 50 ml mark.
8. Mix well. Allow to stand for 10 minutes.
9. Compare the turbidity of the sample to that of the standard against a dark background.
10. If the sample is less turbid than the standard, report as less than 50 ppm.

000132

**NOTE:**

The following altered sample weights may be used to quantify other levels of sulfates:

<u>Sample Weight</u>	<u>Detection Level</u>
1.25	<80 ppm
1.00	<100 ppm

**9. DOCUMENT CHANGE RECORD**

<u>Rev. No.</u>	<u>Eff. Date</u>	<u>Change</u>
0	11/1/97	None - New Work Instruction

**Appendix A**

**Part 2**

**Specifications and analytical protocols**

**000134**



**Specifications and Analytical Protocols**

<b>Parameters</b>	<b>Specification</b>	<b>GPC Analytical method</b>	<b>SPI Analytical method</b>
Dextrose equivalent	<1.0	C-21	9070
%Moisture	10.0% max	C-27	9859
%Solids(liquid product)	50% min	C-27 C-13	9859
Ash	0.1%	C-49	2740
pH	4-7	C-25	
Heavy metals	<10ppm	C-37	5181
Chlorides	50ppm max	C-56	3061
Sulfates	100 ppm max	C-56	9220
Arsenic	<2.5ppm	Analyzed by ICP	Analyzed by ICP

**000135**

The following tables depict typical properties of HSH.

Listed below is the carbohydrate composition of the various hydrogenated starch hydrolysates:

DP Profile	HSH "A"		HSH "B"		HSH "C"		HSH "D"	
DP >8	46.59	± 4%	54.7	± 4%	67.81	± 4%	86.1	± 4%
DP 8	3.93	± 2%	4.8	± 1.5%	4.53	± 1.5%	2.39	± 1%
DP 7	9.52	± 2%	9.1	± 1.5%	7.03	± 1.5%	2.91	± 1%
DP 6	11.37	± 2%	8.4	± 1.5%	6.13	± 1.5%	2.37	± 1%
DP 5	5.91	± 2%	4.7	± 1.5%	3.28	± 1.5%	1.44	± 1%
DP 4	6.35	± 2%	5.5	± 1.5%	3.67	± 1.5%	1.50	± 1%
DP 3	8.28	± 2%	6.7	± 1.5%	4.17	± 1.5%	1.63	± 1%
DP 2	6.19	± 2%	4.8	± 1%	2.53	± 1%	1.04	± 1%
DP 1	1.82	± 1.5%	1.3	± 1%	0.67	± 1%	0.61	± 1%

GPC Standard Analytical method for DP profile is C-46

Analysis	Specification	HSH "A"	HSH "B"	HSH "C"	Analytical method
Dextrose equivalent	<1.0	0.4	0.5	0.4	C-21
Moisture	10.0% max	4.3	3.0	5.0	C-27
Ash	0.1% max	<0.1	<0.1	0.01	C-49
pH	4-7	5.8	6.1	6.9	C-25

Viscosity and pH of Hydrogenated Starch Hydrolysates.

SAMPLE DESCRIPTION	pH	VISCOSITY, cps.*					
		% SOLIDS	at 30C	at 40C	at 50C	at 60C	at 70C
10 DE Based Product	4.51	50	279	202	157	106	81
		40	118	93	75	60	41
18 DE Based Product	4.21	50	77	60	52	49	43
		40	44	35	31	28	24

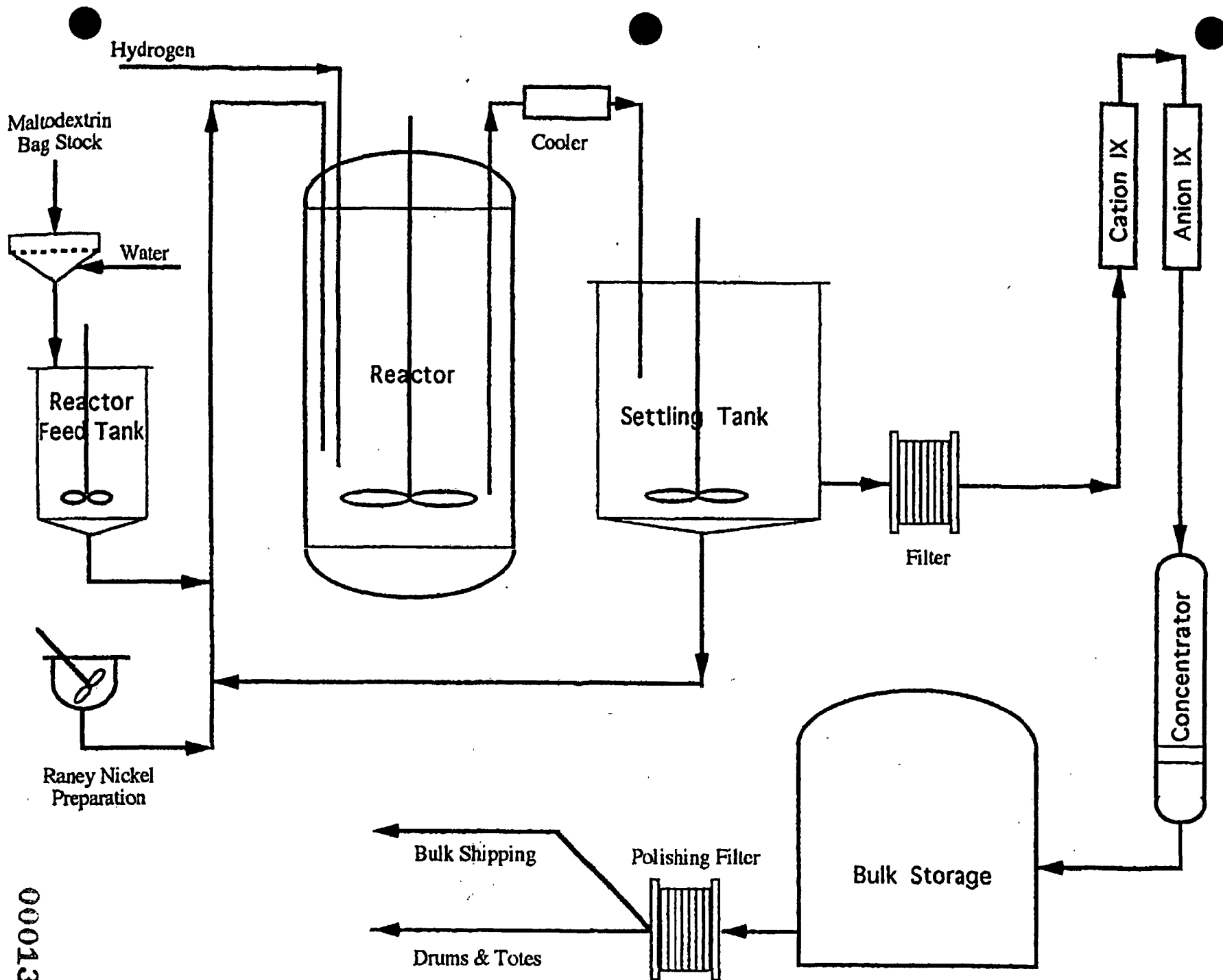
\* Brookfield RVT Viscosity @ 100rpm

000136

## Typical representative chemical composition of starting HSH A raw material

Parameters	Test results
Dextrose equivalent	18
DP 1	1.6
DP 2	5.8
DP 3	7.8
DP 4	6.1
DP 5	5.4
DP 6	10.2
DP 7	10.2
DP8	4.4
>DP8	48.5
Moisture	5
pH	4.0-4.7
Ash	<0.5

**Appendix A**  
**Part 3**  
**Details of Manufacturing Process**



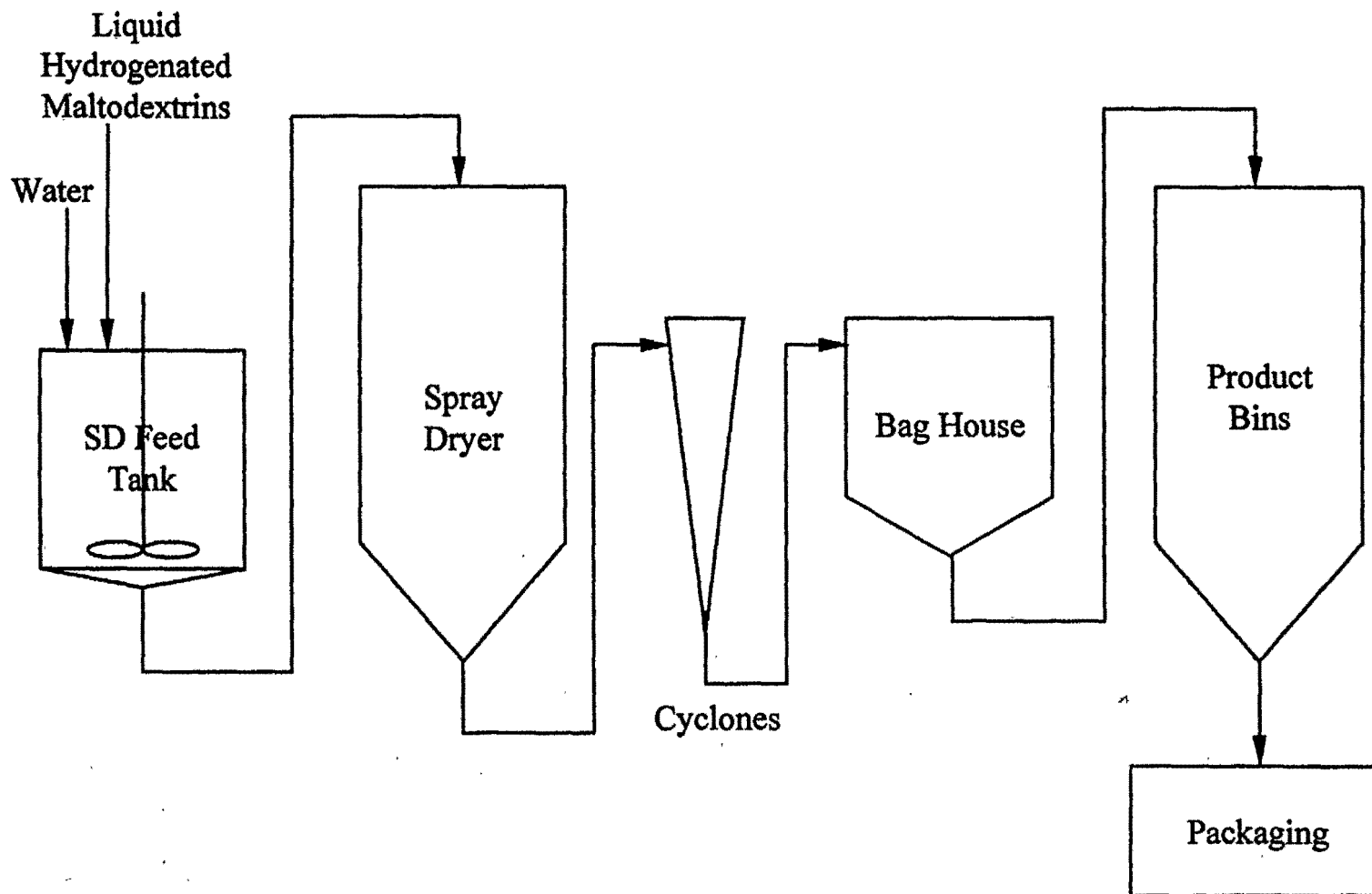
0001339

Index to EIA Response to NHTS Form Request No. 2018-0012 - 0001339

Hydrogenation of Maltodextrins - SPI Polyols, Inc.

Page 13 of 17  
SPEC-02

KL 2/21/00

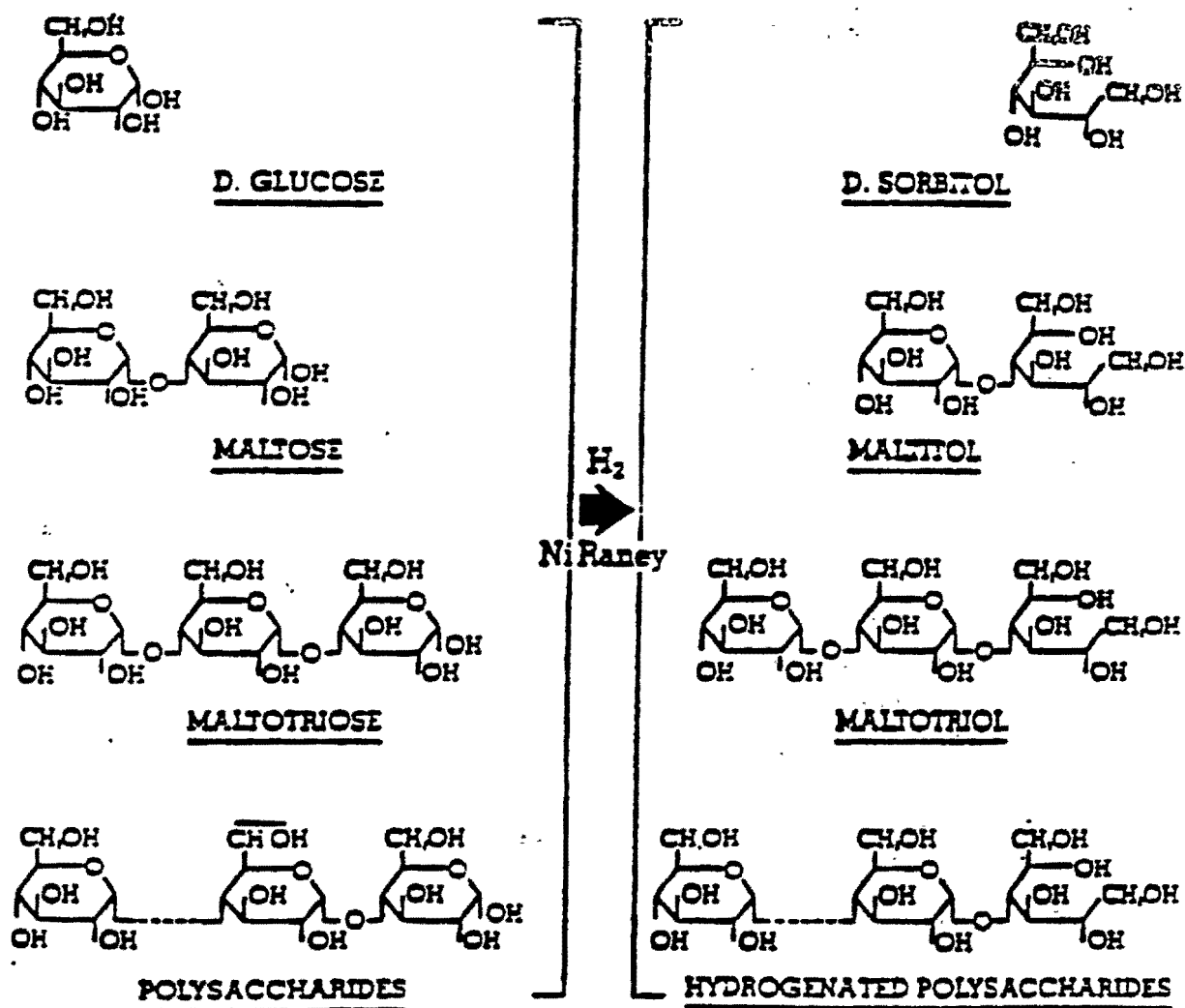


Drying of Hydrogenated Maltodextrins -  
Grain Processing Corporation

MU 3/3/00

# HYDROGENATION

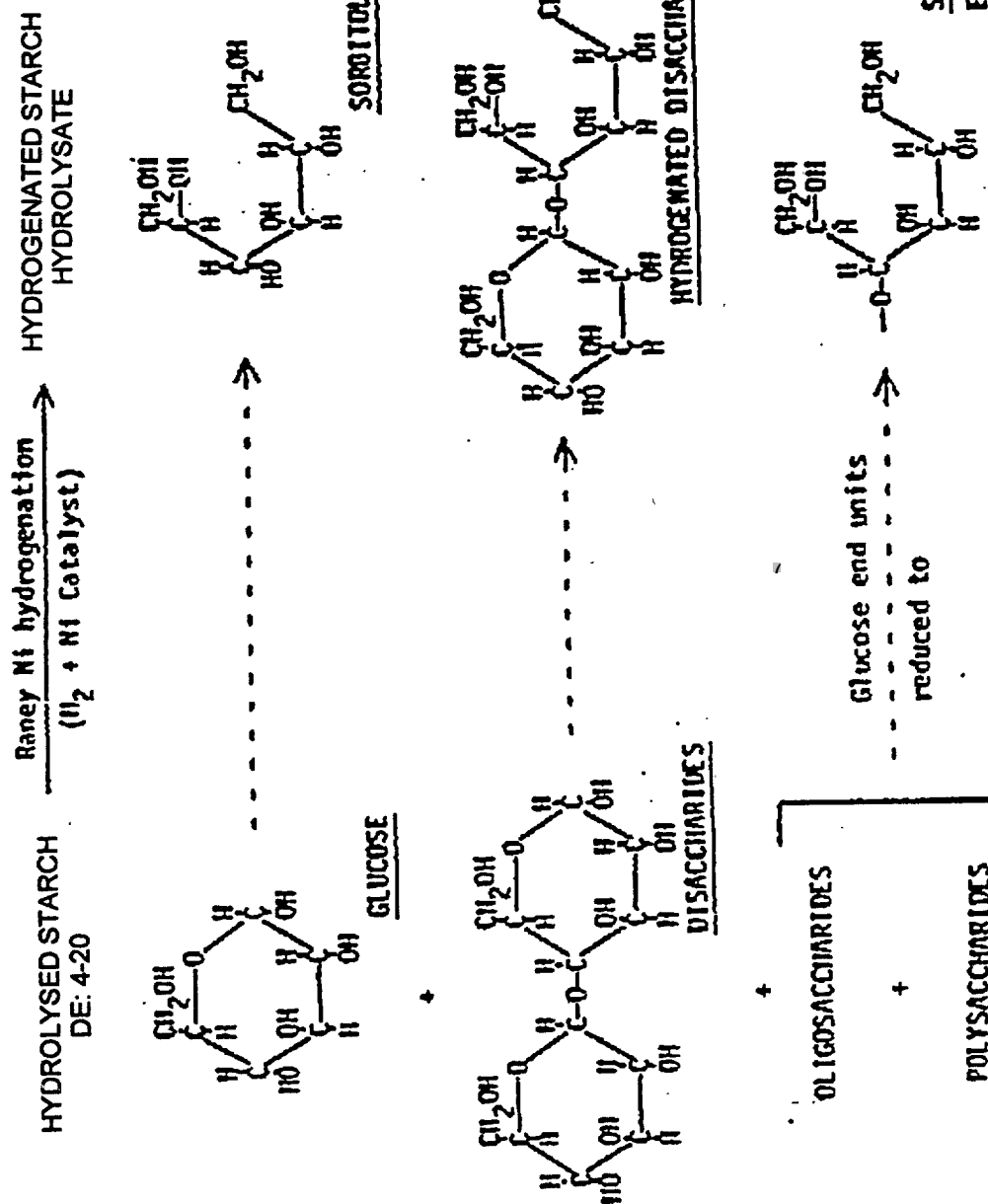
## HYDROGENATED STARCH HYDROLYSATE



000141

# SCHEMATIC PRODUCTION PROCESS

## FOR HYSTARS



000142

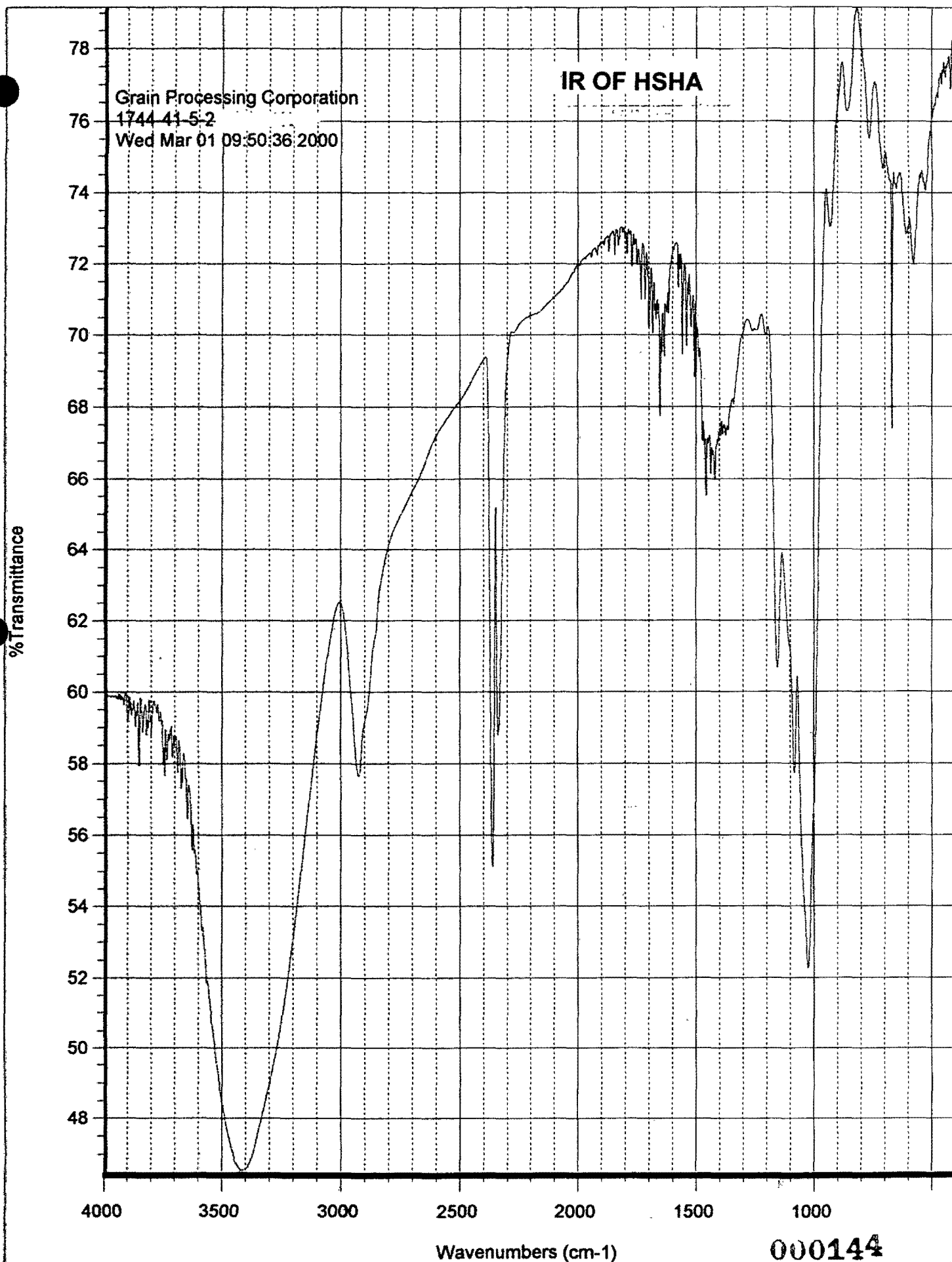


**Appendix A**

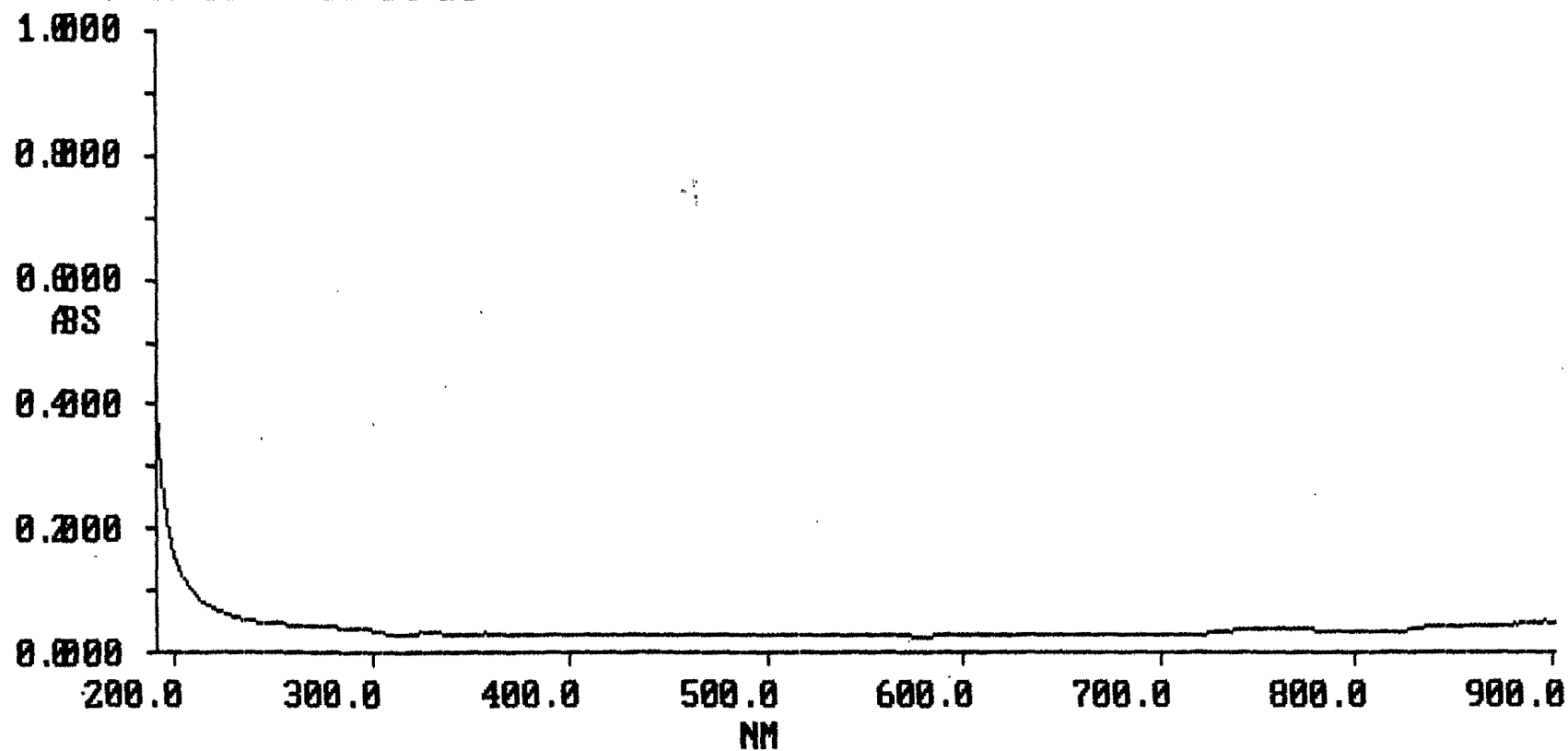
**Part-4**

**Infrared and ultraviolet spectra**

**000143**



X:USER001 ; absc 900.0- 190.0; pts 711; int 1.00; ord 0.0284-0.4414; A  
inf: 17:44:53 99/12/20



000145

**Appendix A**

**Part – 5**

**HPLC Profile of Hydrogenated Starch Hydrolysate.**

**000146**

---

Software Version	: 6.1.0.2:G07	Date	: 10/20/99 02:52:44 PM
Operator	: research2	Sample Name	: 1763-88
Sample Number	: 02	Study	: 4125
AutoSampler	: NONE	Rack/Vial	: 0/0
Instrument Name	: HPLC-CARB	Channel	: A
Interface Serial #	: 5004272419	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 50.00 min
Sampling Rate	: 1.0000 pts/s		
Volume Injected	: 1.000000 uL	Area Reject	: 5000.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 10/20/99 02:01:50 PM	Cycle	: 2

Raw Data File : e:\data\carbohy\1763-88.raw

Result File : E:\DATA\CARBOHY\1763-88.rst

Inst Method : e:\tc4\methods\carbohyt from E:\DATA\CARBOHY\1763-88.rst

Proc Method : e:\tc4\methods\carbohyt from E:\DATA\CARBOHY\1763-88.rst

Calib Method : e:\tc4\methods\carbohyt from E:\DATA\CARBOHY\1763-88.rst

Sequence File : E:\tc4\SEQUENCE\Cart 10-20-99 .seq

Noise Threshold : 34

Area Threshold : 171

Bunch Factor : 4

Multiplier : 1.0000

Divisor : 1.0000

Addend : 0.0000

User1 :

User2 :

User3 :

User4 :

User5 :

User6 :

User7 :

User8 :

User9 :

User10 :

Total number of peaks detected : 12

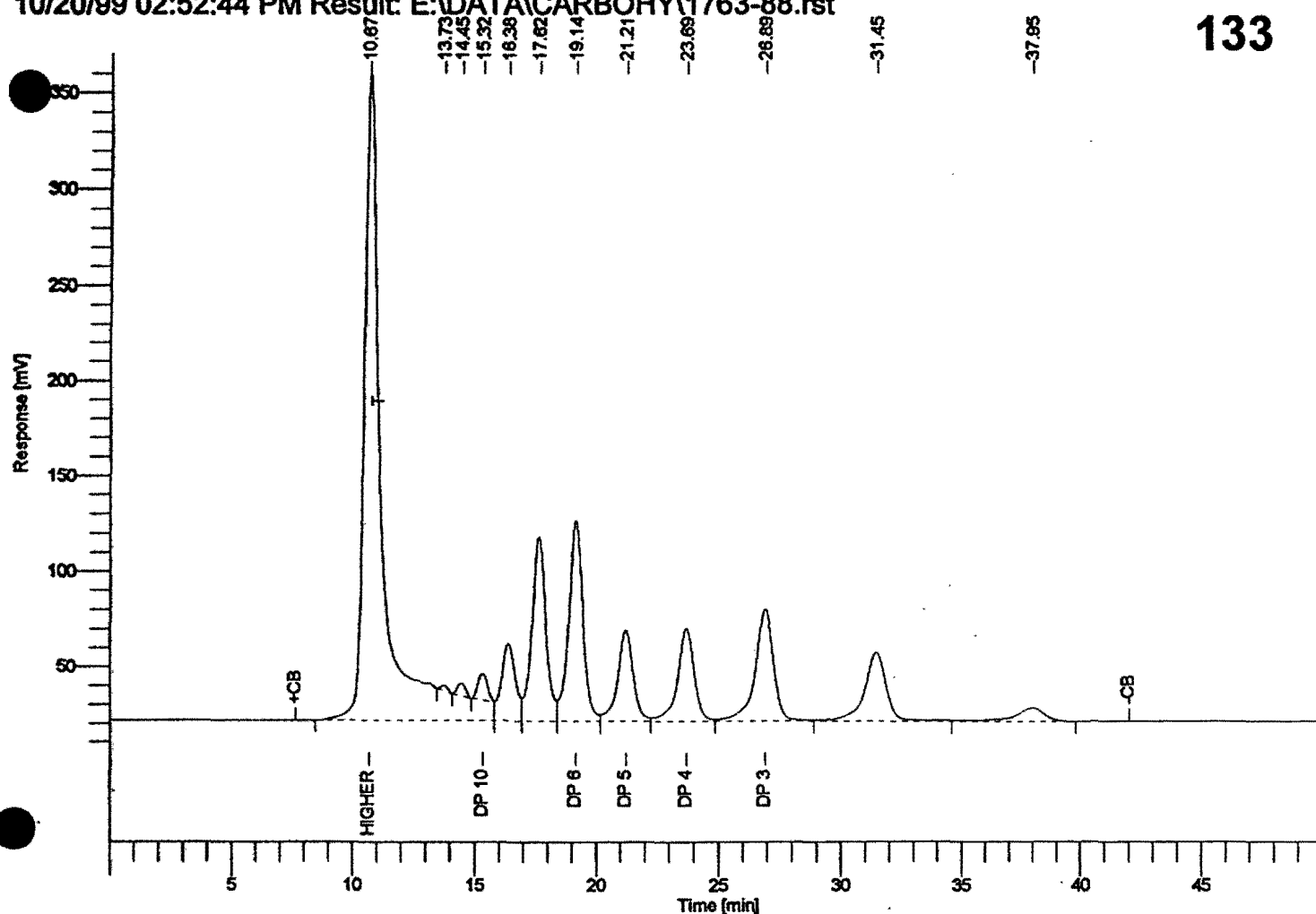
Instrument Conditions

---

000147

10/20/99 02:52:44 PM Result: E:\DATA\CARBOHY\1763-88.rst

133



\*\*\*\*\*CARBOHYDRATE REPORT\*\*\*\*\*

Component Name	Time [min]	BL	Area [μV-s]	Norm. Area [%]
HIGHERS	10.667	*BT	18052746.05	46.30
	13.728	*TT	53696.00	0.14
	14.447	*TT	152046.00	0.39
DP 10	15.316	*TT	376769.00	0.97
	16.375	*VV	1654603.28	4.24
	17.618	*VV	3865562.11	9.91
DP 6	19.143	*VV	4299058.96	11.03
DP 5	21.206	*VV	2145675.83	5.50
DP 4	23.691	*VV	2346479.17	6.02
DP 3	26.889	*VV	3122544.18	8.01
	31.447	*VV	2296638.96	5.89
	37.949	*VB	622468.25	1.60
			38988287.79	100.00

000148

Sample Name : 1763-68  
FileName : c:\data\carbonyl\1763-68.raw  
Date : 10/20/99 02:52:45 PM

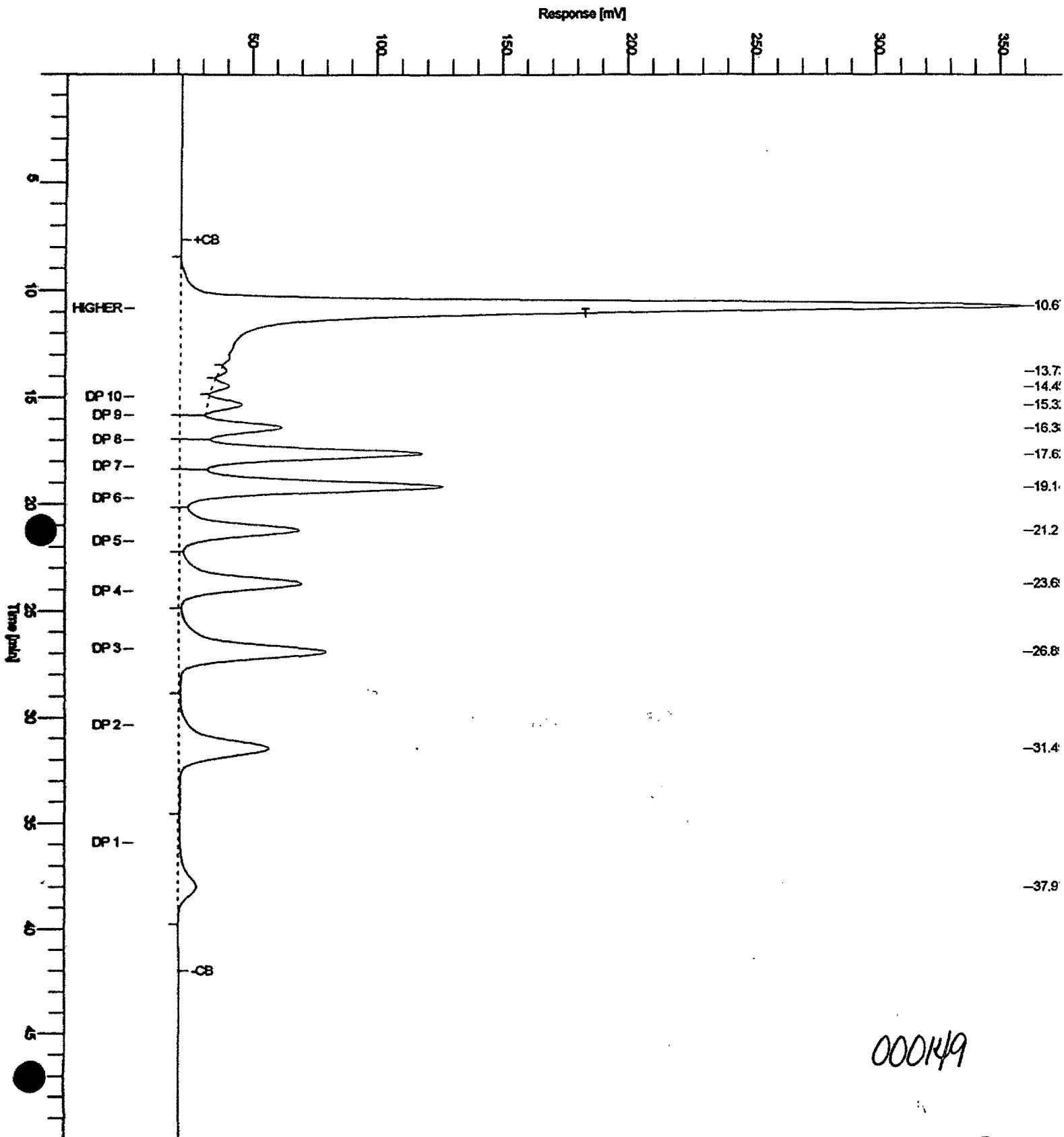
Sample #: 02

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Method :  
Start Time : 0.00 min End Time : 60.00 min Time of Injection: 10/20/99 02:01:50 PM  
Scale Factor: 0.0 Plot Offset: 3.26 mV Low Point : 3.26 mV High Point : 360.03 mV  
Plot Scale: 356.8 mV

# HSH A



Software Version	: 6.1.0.2:G07	Date	: 11/24/98 09:52:46 AM
Operator	: research2	Sample Name	: 1744-36-A
Sample Number	: 02	Study	: 4125
AutoSampler	: NONE	Rack/Vial	: 0/0
Instrument Name	: HPLC-CARB	Channel	: A
Interface Serial #	: 5004272419	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 50.00 min
Sampling Rate	: 1.0000 pts/s	Area Reject	: 5000.000000
Volume Injected	: 1.000000 uL	Dilution Factor	: 1.00
Sample Amount	: 1.0000	Cycle	: 2
Data Acquisition Time	: 11/23/98 11:20:42 AM		

**135**

Raw Data File : e:\data\carbohy\1744-36-A.raw

Result File : E:\DATA\CARBOHY\1744-36-A.rst

Inst Method : e:\tc4\methods\carbohyt from E:\DATA\CARBOHY\1744-36-A.rst

Proc Method : e:\tc4\methods\carbohyt from E:\DATA\CARBOHY\1744-36-A.rst

Calib Method : e:\tc4\methods\carbohyt from E:\DATA\CARBOHY\1744-36-A.rst

Sequence File : E:\tc4\SEQUENCE\Cart 11-23-98 .seq

Noise Threshold : 12

Area Threshold : 62

Bunch Factor : 7

Multiplier : 1.0000

Divisor : 1.0000

Addend : 0.0000

User1 :

User2 :

User3 :

User4 :

User5 :

User6 :

User7 :

User8 :

User9 :

User10 :

Total number of peaks detected : 16

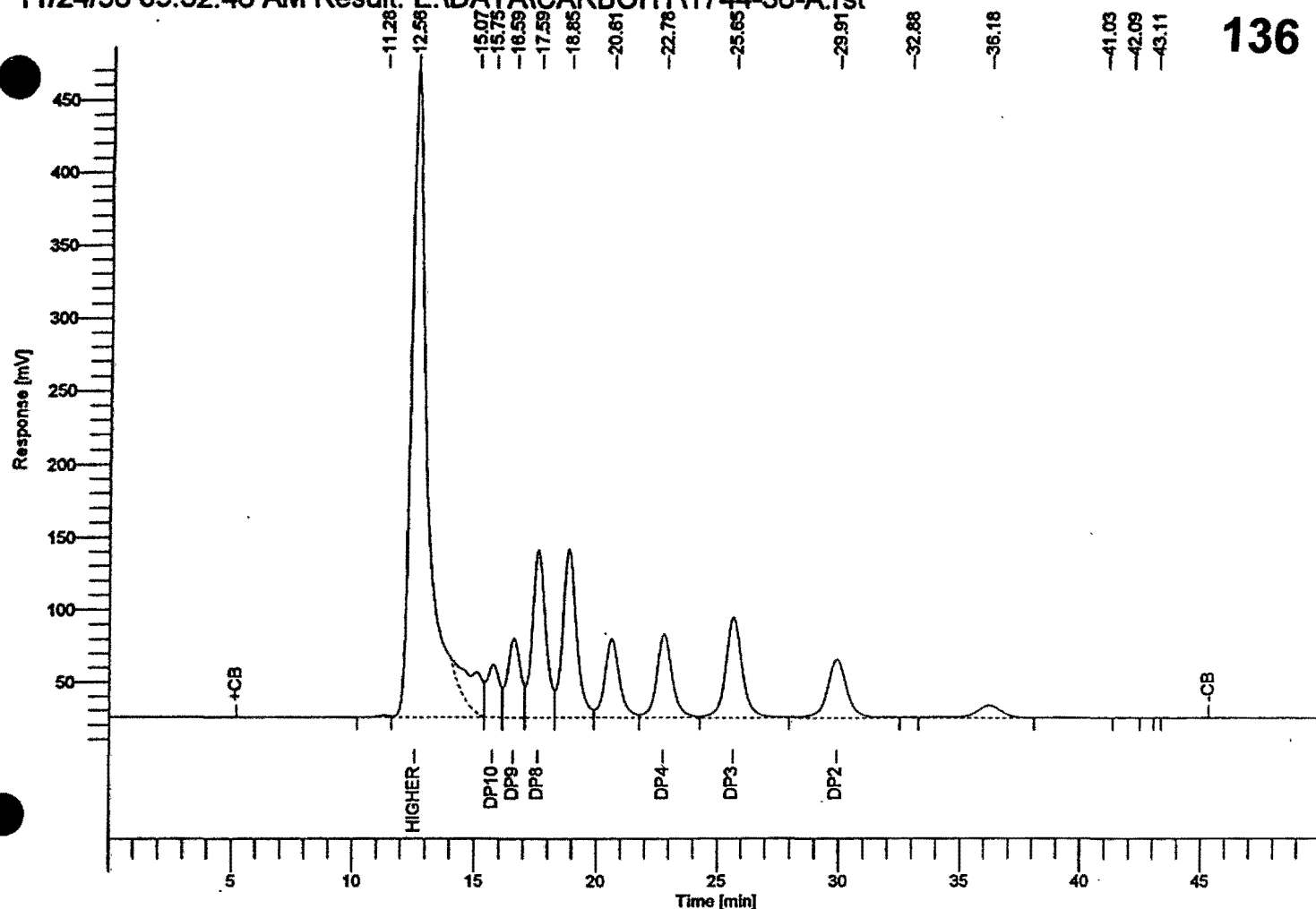
Instrument Conditions

**000150**



11/24/98 09:52:46 AM Result: E:\DATA\CARBOHY\1744-36-A.rst

136



\*\*\*\*\*CARBOHYDRATE REPORT\*\*\*\*\*

Component Name	Time [min]	BL	Area [ $\mu\text{V}\cdot\text{s}$ ]	Norm. Area [%]
HIGHERS	11.284	*BV	46205.09	0.10
	12.559	*VE	21134474.82	45.28
	15.072	*EV	1539032.00	3.30
DP10	15.754	*VV	1315281.42	2.82
DP9	16.592	*VV	2063691.30	4.42
DP8	17.592	*VV	4493613.94	9.63
	18.851	*VV	4715898.64	10.10
	20.610	*VV	2339900.30	5.01
DP4	22.777	*VV	2615078.45	5.60
DP3	25.654	*VV	3362530.49	7.20
DP2	29.908	*VV	2297774.59	4.92
	32.884	*VV	20667.26	0.04
	36.183	*VB	681002.18	1.46
	41.032	*BV	35105.62	0.08
	42.089	*VB	10391.88	0.02
			46670648.00	100.00

000151

Sample Name : 1744-36-A  
FileName : e:\data\carbonyl\1744-36-A.raw  
Date : 11/24/98 09:52:48 AM  
Method :  
Start Time : 0.00 min  
Factor: 0.0

Sample #: 02

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137

Time of Injection: 11/23/98 11:20:42 AM

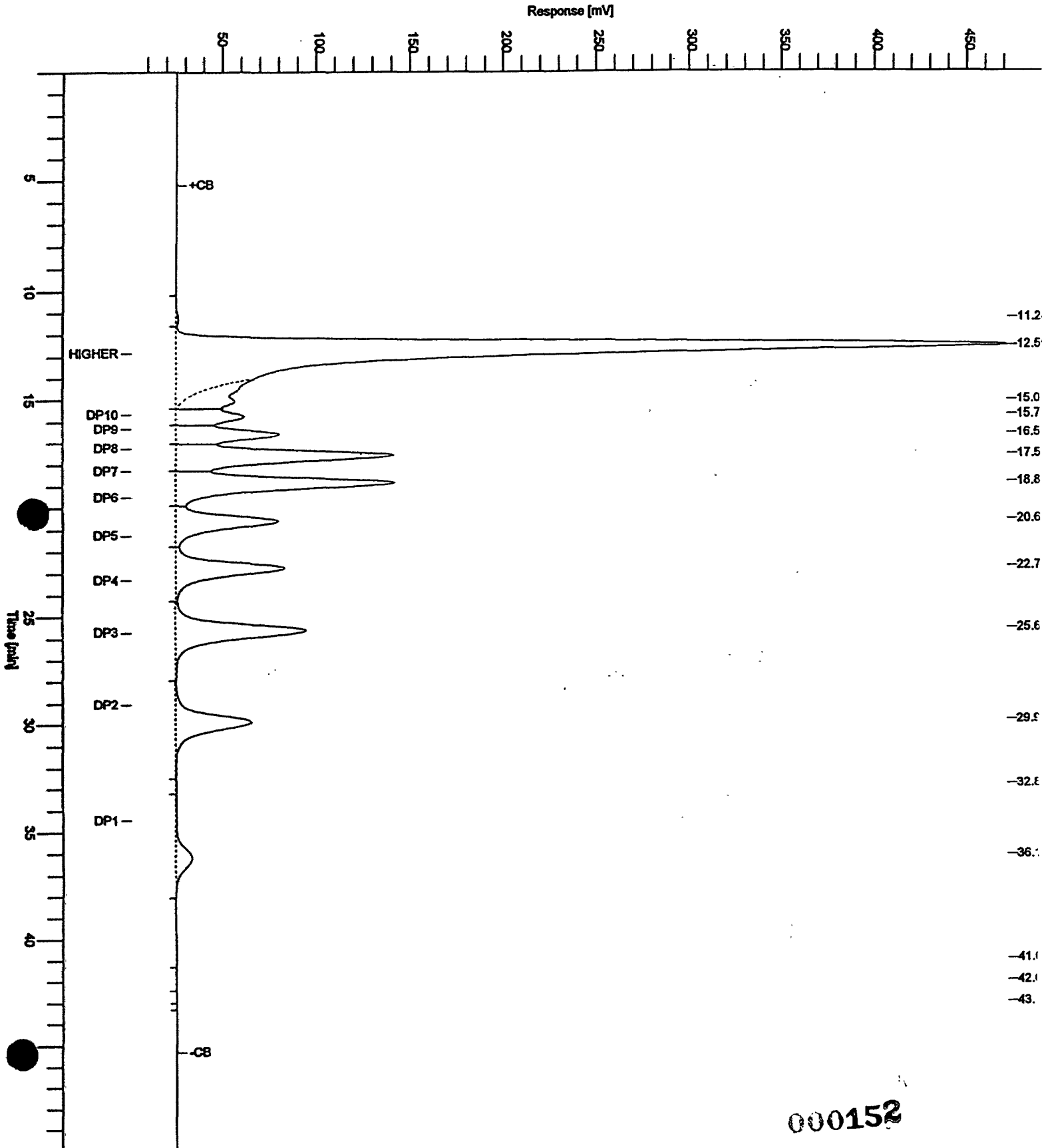
Low Point : 1.63 mV

High Point : 471.65 mV

Plot Offset: 1.63 mV

Plot Scale: 470.0 mV

## HSH B



000152

Software Version	: 6.1.0.2:G07	Date	: 12/30/98 11:07:54 AM
Operator	: research2	Sample Name	: 1744-53-1
Sample Number	: 06	Study	: 4100
AutoSampler	: NONE	Rack/Vial	: 0/0
Instrument Name	: HPLC-CARB	Channel	: A
Interface Serial #	: 5004272419	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 50.00 min
Sampling Rate	: 1.0000 pts/s	Area Reject	: 5000.000000
Volume Injected	: 1.000000 uL	Dilution Factor	: 1.00
Sample Amount	: 1.0000	Cycle	: 6
Data Acquisition Time	: 12/29/98 04:25:16 PM		

**138**

Raw Data File : e:\data\carbohy\1744-53-1.raw  
 Result File : E:\DATA\CARBOHY\1744-53-1.rst  
 Inst Method : e:\tc4\methods\carbohyt from E:\DATA\CARBOHY\1744-53-1.rst  
 Proc Method : e:\tc4\methods\carbohyt from E:\DATA\CARBOHY\1744-53-1.rst  
 Calib Method : e:\tc4\methods\carbohyt from E:\DATA\CARBOHY\1744-53-1.rst  
 Sequence File : E:\tc4\SEQUENCE\Cart12-29-98.seq

Noise Threshold	: 12	Area Threshold	: 62
Bunch Factor	: 7	Multiplier	: 1.0000
Divisor	: 1.0000	Addend	: 0.0000

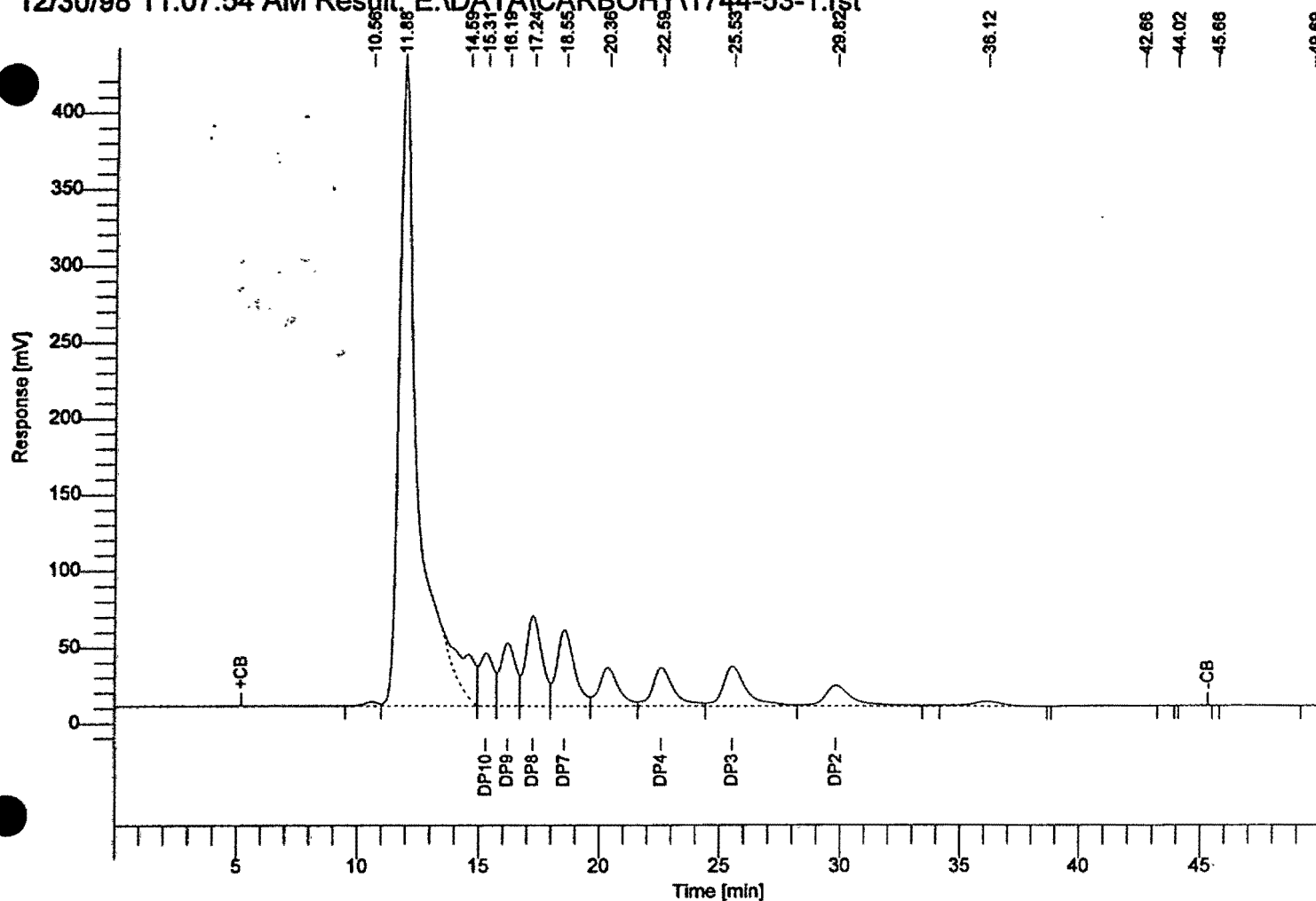
User1 :  
 User2 :  
 User3 :  
 User4 :  
 User5 :  
 User6 :  
 User7 :  
 User8 :  
 User9 :  
 User10 :

Total number of peaks detected : 16

Instrument Conditions

**000153**

12/30/98 11:07:54 AM Result: E:\DATA\CARBOHY\1744-53-1.rst



\*\*\*\*\*CARBOHYDRATE REPORT\*\*\*\*\*

Component Name	Time [min]	BL	Area [μV·s]	Norm. Area [%]
	10.559	*BV	104779.08	0.27
	11.881	*VE	22712557.39	58.22
	14.588	*EV	1557005.00	3.99
DP10	15.308	*VV	1397682.79	3.58
DP9	16.192	*VV	1810412.21	4.64
DP8	17.240	*VV	2802080.35	7.18
DP7	18.548	*VV	2551056.65	6.54
	20.356	*VV	1395347.17	3.58
DP4	22.588	*VV	1524339.32	3.91
DP3	25.528	*VV	1706746.40	4.37
DP2	29.825	*VV	1074386.17	2.75
	36.116	*VB	302836.84	0.78
	42.656	*BB	58462.50	0.15
	49.691	BB	14823.00	0.04
			39012514.87	100.00

000154

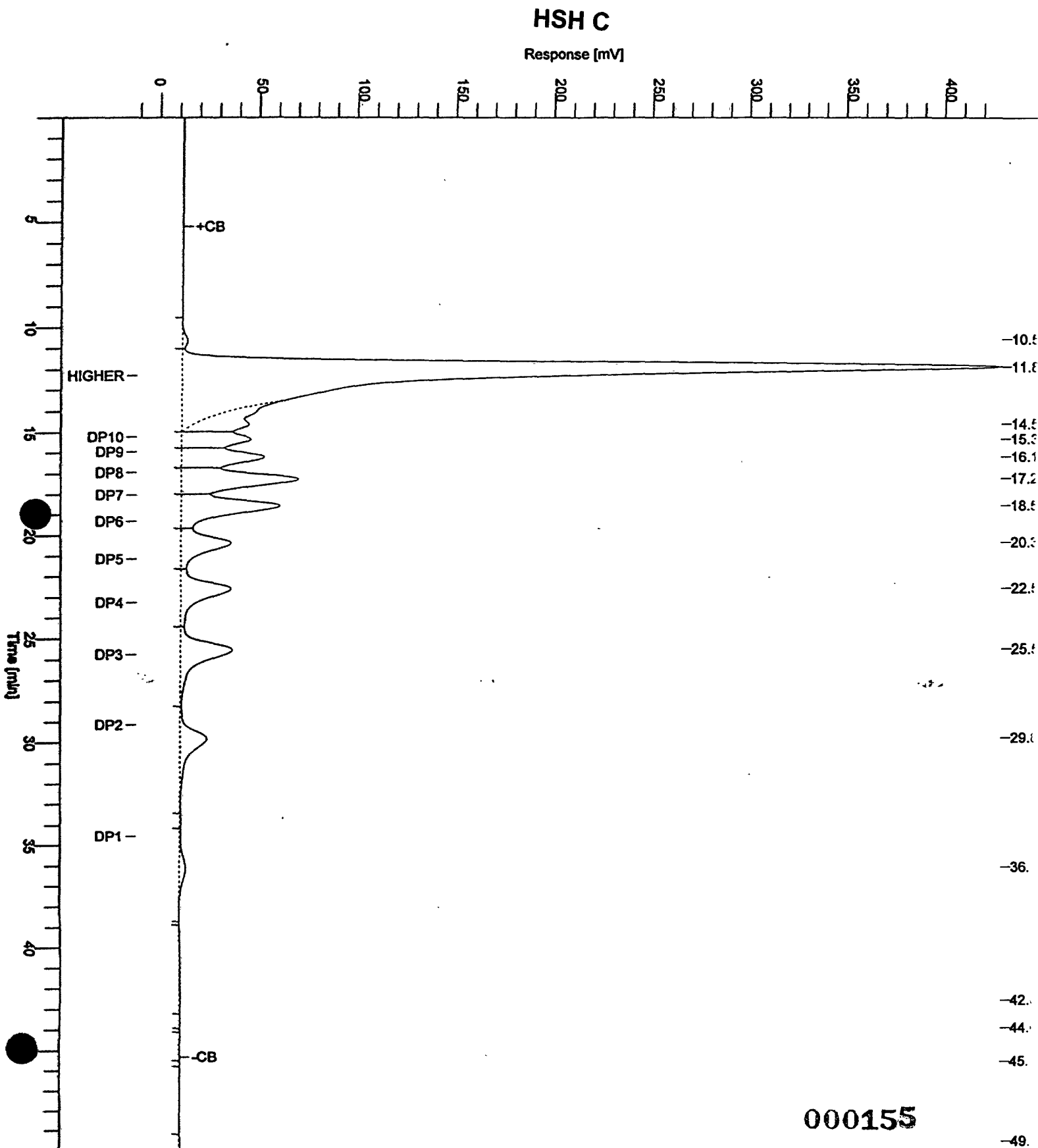
Sample Name : 1744-53-1  
FileName : e:\data\carbonyl\1744-53-1.raw  
Date : 12/30/98 11:07:56 AM

Sample #: 06

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Method :  
Time : 0.00 min End Time : 50.00 min Time of Injection: 12/29/98 04:25:16 PM  
Factor: 0.0 Low Point : -11.30 mV High Point : 429.63 mV  
Plot Offset: -11.30 mV Plot Scale: 440.9 mV



---

Software Version	: 6.1.0.2:G07	Date	: 11/05/99 03:16:08 PM
Operator	: research2	Sample Name	: 1731-73+46-49+34-36 BLEND
Sample Number	: 02	Study	: 4125
AutoSampler	: NONE	Rack/Vial	: 0/0
Instrument Name	: HPLC-CARB	Channel	: A
Interface Serial #	: 5004272419	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 50.00 min
Sampling Rate	: 1.0000 pts/s		
Volume Injected	: 1.000000 uL	Area Reject	: 5000.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 11/05/99 02:23:24 PM	Cycle	: 2

Raw Data File : e:\data\carbohy\1731-73+46-49+34-36 BLEND.raw

Result File : E:\DATA\CARBOHY\1731-73+46-49+34-36 BLEND.rst

Inst Method : e:\tc4\methods\carbohyt from E:\DATA\CARBOHY\1731-73+46-49+34-36 BLEND.rst

Proc Method : e:\tc4\methods\carbohyt from E:\DATA\CARBOHY\1731-73+46-49+34-36 BLEND.rst

Calib Method : e:\tc4\methods\carbohyt from E:\DATA\CARBOHY\1731-73+46-49+34-36 BLEND.rst

Sequence File : E:\tc4\SEQUENCE\Cart 11-05-99 .seq

Noise Threshold : 34

Area Threshold : 171

Bunch Factor : 4

Multiplier : 1.0000

Divisor : 1.0000

Addend : 0.0000

User1 :

User2 :

User3 :

User4 :

User5 :

User6 :

User7 :

User8 :

User9 :

User10 :

Total number of peaks detected : 13

Instrument Conditions

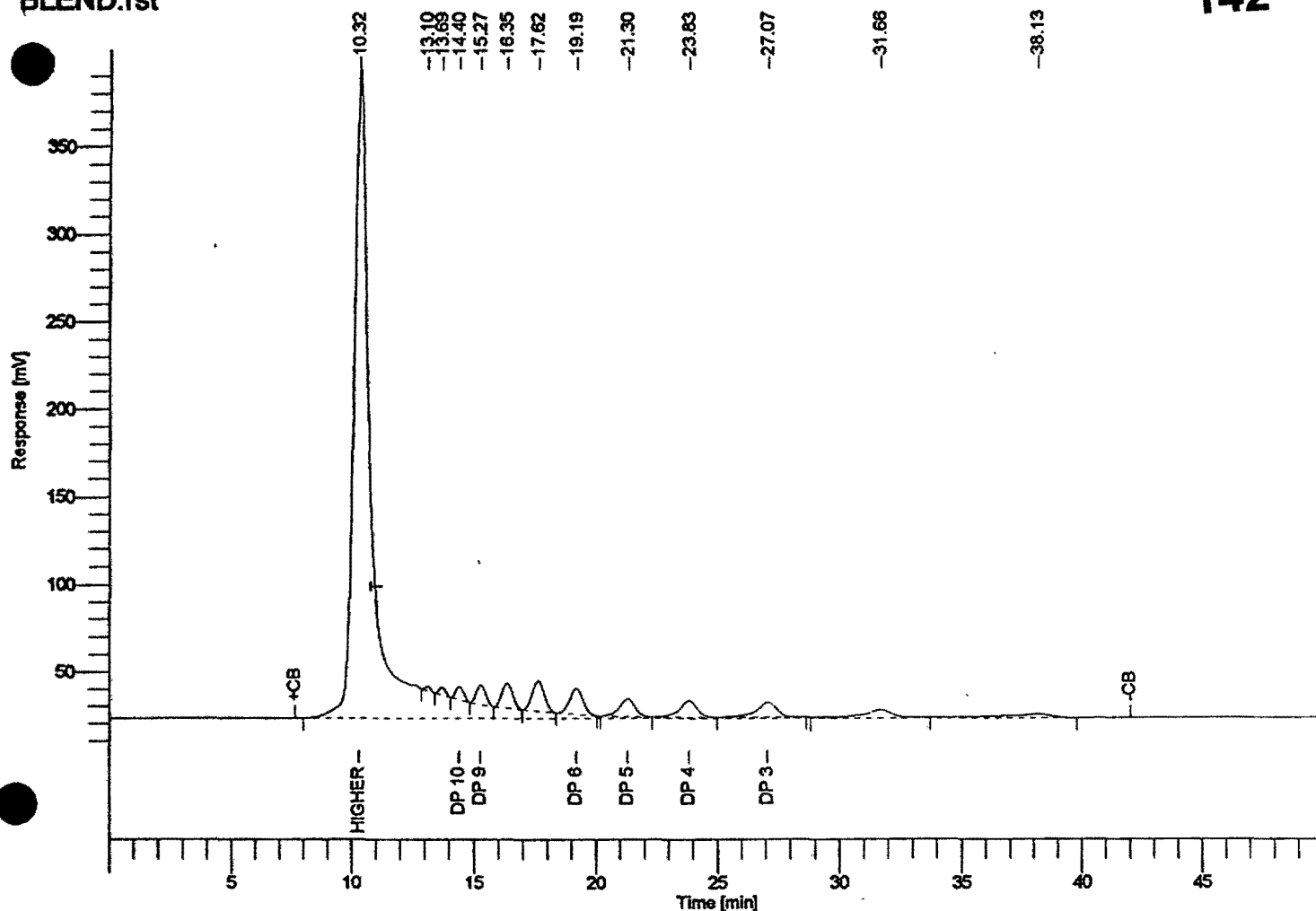
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000156

11/05/99 03:16:08 PM Result: E:\DATA\CARBOHY\1731-73+46-49+34-36

BLEND.rst

142

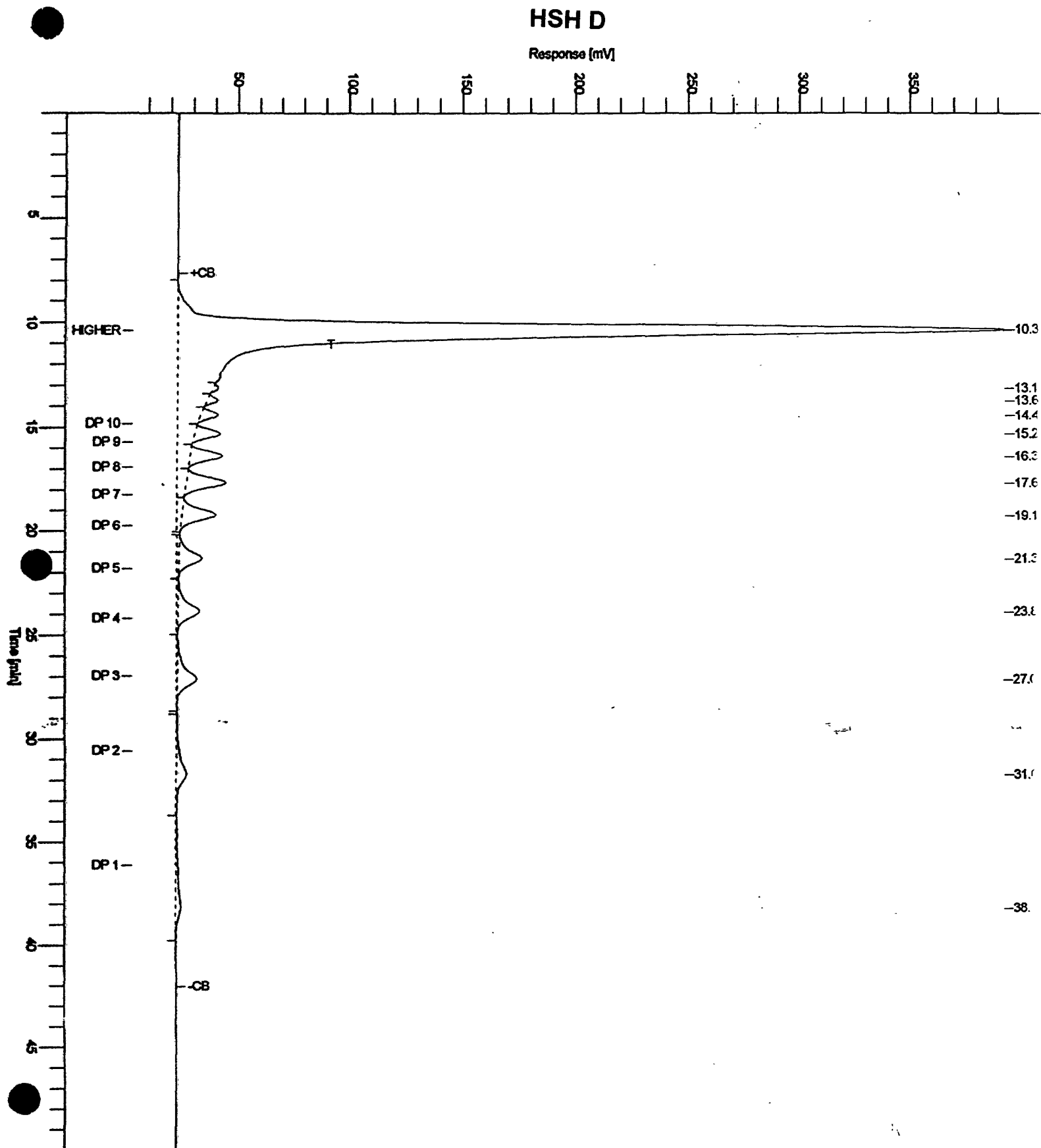


\*\*\*\*\* CARBOHYDRATE REPORT \*\*\*\*\*

Component Name	Time [min]	BL	Area [μV·s]	Norm. Area [%]
HIGHERS	10.315	*BT	21157543.76	82.68
	13.098	*TT	37844.00	0.15
	13.690	*TT	89530.00	0.35
DP 10	14.400	*TT	180922.00	0.71
DP 9	15.274	*TT	311161.00	1.22
	16.346	*TT	447121.00	1.75
	17.621	*TT	616607.00	2.41
DP 6	19.188	*TT	585722.00	2.29
DP 5	21.299	*TT	455727.00	1.78
DP 4	23.829	*TT	482016.00	1.88
DP 3	27.067	*TT	500483.00	1.96
	31.661	*VV	405407.38	1.58
	38.126	*VB	319922.53	1.25
			25590006.67	100.00

000157

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000158



# Appendix B

000159

## **Appendix B**

### **Typical formulas using Hydrogenated Starch Hydrolysate**

The following are typical formulations using HSH for the preparation of the above mentioned food uses. It should be noted that in many cases HSH is used with sugar in the final product, therefore the purpose of this notification is to recommend HSH not as a substitute for sugar but as an adjunct, which improves flavor, increases solids and extends shelf life. With this information, the following recipes offer typical formulas using HSH.

000161

## NO SUCROSE ADDED YELLOW CAKE

<u>Ingredients</u>	<u>Percent by weight</u>
(A) Hydrogenated Starch Hydrolysate (HSH)	26.80
Cake Flour	25.40
Water	14.85
Creamtex (Durkee)	12.00
Whole Milk Powder	0.65
Salt	0.60
 (B) Water	 4.70
 (C) Water	 10.00
Whole Egg Powder (Henningesen)	3.50
Baking Powder	1.20
Vanilla Extract (2X)	0.30

### Procedure

1. Withhold water and blend dry ingredients in part (A).
2. Slowly add water from part (A) to dry ingredients and mix for 5 to 6 minutes at speed #2 in Hobart mixer. Scrape bowl.
3. Add part (B) and mix for an additional 3 minutes.
4. Add part (C) and mix for 4 minutes. Scrape bowl.
5. Bake 450 grams in an 8 x 8 inch pan at 350°F for 30 minutes.

000162

## NO SUCROSE ADDED PANCAKE SYRUP

<u>Ingredients</u>	<u>Percent by weight</u>
Water	50.277
Hydrogenated Starch Hydrolysate (HSH)	48.720
CMC 7H3SF (Hercules)	0.450
Maple Flavor	0.273
Citric Acid	0.075
Sodium Benzoate	0.060
Potassium Sorbate	0.060
Caramel Color	0.045
Sodium Hexametaphosphate	0.040

### Procedure

1. Dissolve CMC in water at room temperature, using a dispersator creating a high speed vortex.
2. Once dissolved, dispersate for 15 minutes.
3. Add Stabilite to CMC/water mixture and disperse while increasing the temperature to 120° F.
4. Once this temperature is reached, dispersate for 10 minutes.
5. Add other ingredients to HSH/CMC/water mixture using a propeller mixer at high speed while increasing the temperature to 180°F.
6. Add ingredients in the following order: citric acid, sodium benzoate, potassium sorbate, sodium hexametaphosphate, flavor, color.
7. Mix at 180 °F for 15 minutes.
8. Place in 180° F water bath for 15 minutes.
9. Cool to room temperature.

000163

**NO SUCROSE ADDED ICING**

<u>Ingredients</u>	<u>Percent by weight</u>
Hydrogenated Starch Hydrolysate (HSH)	5.0
Crystalline Maltitol	26.5
Crystalline Sorbitol	26.5
Shortening with emulsifier	8.0
Unsweetened Chocolate	17.2
Flavor	0.4
Water	16.4

**Procedure**

1. Combine HSH, shortening and water. Bring to a boil.
2. Sift and combine crystalline maltitol and crystalline sorbitol in a Hobart mixer.
3. Using a paddle attachment, mix the crystalline powders on speed #1. While mixing, add heated water mixture.
4. Blend until smooth.
5. Blend in the flavor.
6. Cool to room temperature.

**000164**

## SUCROSE-FREE HARD CANDY

<u>Ingredients</u>	<u>Percent by weight</u>
Stabilite	98.3
Citric Acid	0.5
Flavor	1.0
Color	0.2

### Procedure

1. Cook Stabilite in an open fire cooker to 157 - 160 °C.
2. Pour cooked syrup onto a cooling table.
3. Cool the batch evenly using cool water circulating in the jacket of the cooling table.
4. Continue to cool to 95 - 100°C and then fold in citric acid, flavor and color.
5. While still pliable, form a rope and cut into pieces.
6. Wrap candy and store at room temperature.

**000165**

# Appendix C

APPENDIX C



**Appendix C**

**POTENTIAL PER CAPITA CONSUMPTION OF  
HYDROGENATED STARCH HYDROLYSATE (HSH)**

**000167**

**POTENTIAL PER CAPITA CONSUMPTION OF HYDROGENATED STARCH  
HYDROLYSATE (HSH)**

<b>Food</b>	<b>HSH level</b>	<b>Per Capita Annual Consumption (lbs)</b>	<b>Annual HSH Consumption (lbs)</b>	<b>Daily HSH Consumption (lbs)</b>	<b>Daily HSH Consumption (gm)</b>
*Confections	10-100%	24.8	2.48-24.8	0.006-0.068	0.45-4.99
**Cookies	0%-25%	12.4	0.00-3.10	0.000-0.009	0.00-2.72
**Cakes	0-10%	8.34	0.00-0.80	0.000-0.002	0.00-1.36
**Sweet goods	0-10%	9.06	0.00-0.90	0.000-0.002	0.00-1.81
**Donuts	0-2%	3.11	0.00-0.06	0.000-0.0002	0.00-4.08
*Carbonated Soft Drinks	0-1%	440	0.00-4.40	0.000-0.012	0.00-4.08
***Ice Cream	0-15%	32	0.00-4.80	0.000-0.013	0.00-5.89

\*USDA/Economic Research Service and U.S Department of Commerce, Per capita consumption '97

\*\* U.S Census Bureau 1997 Economic Census

\*\*\*Prepared Foods July 1998

000168

# Appendix D

APPENDIX D

000169

**Appendix D**

**Methods of detecting hydrogenated starch hydrolysate in food.**

000170

## Maltitol: % Assay by Liquid Chromatography

### 1. SCOPE

This method is intended for the determination of Maltitol and Sorbitol via HPLC calibrated with an external standard. An Assurance Test Sample (ATS) is used as a secondary calibration standard on a daily basis. This ATS is quantified monthly vs. USP primary standards for traceability. All results are reported on a dry basis.

### 2. PRINCIPLE

An aqueous solution is passed through a cation exchange column in the lead form, USP packing type L34. Deionized water is used as the eluent and complete resolution of Maltitol and Sorbitol can be expected. The mechanism of this separation is not entirely known, though it is considered to be a combination of weak ion exchange and adsorption chromatography.

### 3. REFERENCES

"Maltitol, HPLC Assay Validation", SPI Polyols Inc., New Castle DE, (Internal Document)

### 4. SAFETY

Refer to the Material Safety Data Sheets (MSDS) for information pertaining to the hazards, handling and disposal of the chemicals used in this method.

### 5. EQUIPMENT

Balance, analytical, capable of weighing to 0.1 mg.

Column – Aminex Fast Carbohydrate Analytical Column, 100 x 7.8 mm, 9 $\mu$ , lead ionic form stationary phase on sulfonated divinyl benzene-styrene copolymer, BioRad P/N 125-0105.

#### WARNING

Analytical methods involve potentially hazardous chemicals, analytical procedures and equipment, and in using these methods you assume all risk of personal injury and property damage. Information about the nature of these hazards and related safety precautions should be obtained before such chemicals, procedures and equipment are used. It is the responsibility of the user to take appropriate precautions.

HPLC system – Hewlett Packard HP1100 or equivalent, controlled by ChemStation, rev. 06.04, software or equivalent. The system consists of the following components:

Isocratic Pump - Hewlett Packard model number G1310A

Heated Column Compartment – Hewlett Packard model G1316A

Autosampler – Hewlett Packard model number G1313A

Refractive Index Detector – Hewlett Packard model number G1362A, 1047A or equivalent.

Vacuum Degasser – Hewlett Packard model number G1322A

Syringes, B-D, 5cc size , or equivalent.

Syringe filters, 0.45µm, 25mm, Millipore part number SLHA02510, or equivalent.

Sample Preparation Vials, 6 dram size.

Autosampler Vials, 1.0 ml, crimp top, Ohio Valley Specialty Chemicals P/N 25175C.

Crimp Caps for autosampler vials, Ohio Specialty Chemicals P/N 14211.

## 6. CHEMICALS

Maltitol, USP Reference Standard.

Sorbitol, USP Reference Standard.

Current Assurance Test Sample.

## 7. REAGENTS

LC mobile Phase

Deionized water is passed through a Type-I Reagent Grade water system, Millipore Milli-Q or equivalent, which has 0.22µm membrane filtration. The water is then transferred to the HPLC solvent reservoir. The water is passed through a vacuum degasser prior to being pumped through the HPLC system.

## 8. STANDARDIZATION

### A. HPLC Instrument Operating Parameters (see Appendix A)

Injection Volume	10 $\mu$ L
Column Temperature	60°C
Detector Temperature	35°C
Flow Rate	0.5 mL/min.
Run Time	25 minutes.

### B. Monthly ATS Standardization with USP Primary Standards

1. Accurately weigh 0.4 g of ATS and dilute to a final weight of 20g with Type-I water, record all weights to the nearest 0.1 mg and mix.
2. Accurately weigh 0.05 g USP Sorbitol RS and 0.05 g USP Maltitol RS into a sample preparation vial. Dilute to 10 g final weight using Type-I water. Record all weights to the nearest 0.1 mg and mix.
3. Filter the ATS and USP RS samples into autosampler vials using a 5cc syringe with a syringe filter attached. Note: the proper method for installing a syringe filter is to remove the plunger from the syringe barrel prior to attaching the filter cartridge. Drawing the plunger completely out of the barrel while the cartridge is attached may cause tearing of the filter membrane.
4. Load the appropriate analytical method into the ChemStation software and enter the sample weights, dilutions and concentrations into the appropriate fields of the sequence table and calibration table. Note: The pathname for this method is C:\HPCHEM\instrument number\CALIB\MALTITOL.M
5. Calibrate the method with two replicate injections of the USP RS mixture. Replacing the existing calibration table with the first calibration and average in the second. In the *Data Analysis* section of ChemStation, select the report style of *Performance*. Print out the Calibration chromatograms and save the hard copies in a file for system monitoring and evaluation with respect to performance characteristics such as peak symmetry, resolution, plate count and relative retention times.
6. Quantify the ATS solutions with the new calibration table and record the values in the Primary Calibration notebook.
7. If the ATS quantifies at the labeled values  $\pm 0.25\%$ , record the value and proceed with the analysis. If the ATS quantifies outside of the above tolerance range than rerun this entire section using new calibration solutions and new ATS workups. If the ATS still fails to quantify within the above tolerances, consult the chemist on duty.

000173

C. Daily calibration using the current ATS

1. Using an ATS sample prepared as in B-1, load the appropriate method and sample information into the ChemStation software and perform replicate calibrations, as per B-4 thru B-5. Note: The pathname for this method is C:\HPCHEM\“instrument number”\METHODS\MALTITOL.M

9. Analysis of Production Samples, Procedure.

1. Accurately weigh duplicate weights of 0.4 g each of production sample into separate sample vials, dilute each to 20g with Type-I water and mix. Record all weights to the nearest 0.1 mg.
2. Filter the sample into an autosampler vial using a syringe and syringe filter, see note section 8, B-3.
3. Using the ChemStation software package, load the analytical method calibrated above.
4. Setup a sequence table which has two daily ATS samples, called “Daily ATS #1” & “Daily ATS #2” prior to production samples (lead ATS’s). Include a weekly ATS sample along with a duplicate of “Daily ATS #1” after the production replicates (follow up ATS’s). Comparison of Daily ATS #1 lead and follow up indicates system stability during the run, tracking the weekly follow up shows long term stability and/or trends. The weekly ATS will also be used to assess continuous system suitability as per the process instruction for chromatographic system suitability. Differences in assay between the two daily lead ATS’s is an indicator of possible work-up error.
5. Enter the sample name, the sample weight and the final weight of the solution in the sequence table as *Sample Name*, *Sample Amount* and *Dilution* respectively. Also, in order to report final results on a dry basis, divide one by the percent solids and enter this value into the sequence table as *Multiplier*.
6. Compare the ATS results with the appropriate SQC chart, if all of the ATS values are within the specified ranges of the SQC chart, the production analysis is considered to have been collected properly. The results are entered into LIMS and the follow up daily ATS assay value is recorded on the appropriate SQC chart. If the ATS assay values are out of the specified range a fresh set of ATS’s must be worked up and the sequence will be rerun using the new ATS’s. If the ATS values still fail to fall within the specified ranges, consult with the chemist on duty.
7. The software package will calculate the component values based upon the existing calibration table.

10. Calibrations and Software Settings

As noted above, the ChemStation software will perform all the necessary calculations. The report style should be set to *Short*; %ESTD and Based On *Area* should also be selected.



Resolution and other column performance statistics may be accessed by changing the report style to *Performance* and reprinting the chromatogram.

### 11. Calculations

Component Assay values can be calculated manually with the following equation:

$$\% \text{Component (as is)} = \frac{\text{Area of Component} \times \text{RF} \times \text{Solution Final Wt.}}{\text{Sample Weight}} \times 100\%$$

$$\% \text{Component (db)} = \frac{\% \text{Component (as is)}}{\text{Percent Solids}}$$

Where RF is the response factor determined during the calibration as:

$$\text{RF} = \frac{\text{Concentration of Analyte}}{\text{Area of Analyte Peak}}$$

### 12. Document Change Record

<u>Rev. No.</u>	<u>Eff. Date</u>	<u>Change</u>
1	8/1/99	•Chromatographic system and conditions changed. Sample preparation changed. ATS/SQC procedure included.

000175

**Maltitol Solution**  
**HPLC Assay Validation**

Prepared By:

~~Paul M. [Signature]~~  
Chemist

Date:

10/13/99

Approved By:

~~[Signature]~~  
Laboratory Manager

Date:

10/13/99

**Rationale:**

This assay method was specifically developed to allow for the quantification of the primary components in Maltitol Solutions; maltitol and sorbitol. The method is a part of a broader effort to unify the analysis of several USP/NF polyols.

**Analytical Procedure**

*Mobil phase*- Use degassed water.

*Standard preparation* - Dissolve USP Maltitol RS and USP Sorbitol RS in water to obtain a solution having concentrations of about 10 mg/g and 1.6 mg/g respectively.

*Assay preparation*, - Accurately weigh 0.4 g of Maltitol Solution, record to the nearest 0.1 mg, dilute with water to about 20 g, record the final solution weight to the nearest 0.1 mg, and mix thoroughly.

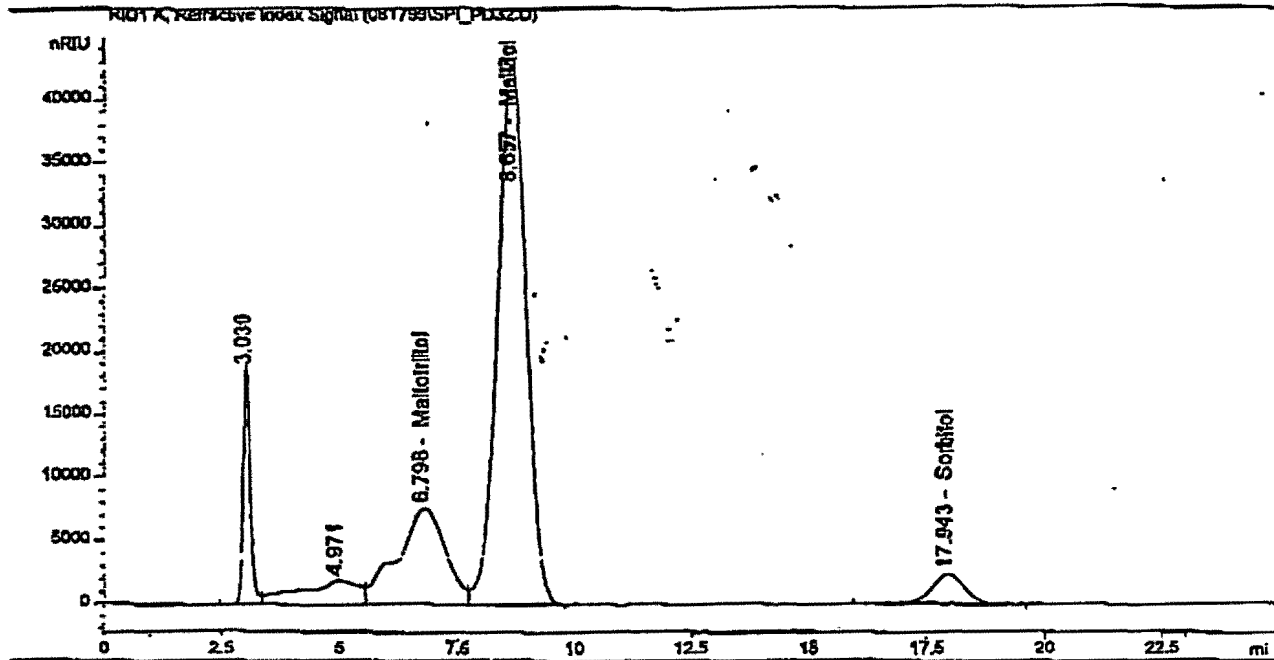
*Chromatographic system* (see *Chromatography* <621>)- The liquid chromatograph is equipped with a refractive index detector maintained at a constant temperature of about 35°C, and a 7.8-mm x 10-cm analytical column containing packing L34. The column is maintained at a constant temperature of about 60°C controlled within +/- 2.0 °C and the flow rate is about 0.5 mL per minute. Chromatograph 10 µL of the *Standard preparation*, and record the peak responses as directed under *Procedure*. The tailing factor for maltitol and sorbitol is not more than 1.2 with a maximum RSD of 2.0% for replicate injections.

*Procedure*- Separately inject equal volumes (about 10 µL) of the *Standard preparation*, and the *Assay preparation* into the chromatograph, record the chromatograms and measure the responses for the major peaks. The relative retention times are about 0.38 for maltotriitol, 0.48 for maltitol and 1.0 for sorbitol. Separately calculate the quantities, in %, of maltitol and sorbitol in the portion of Maltitol Solution sample, taken by the formula:

$$(C_u/C_s)(r_u/r_s) \times 100$$

in which  $C_u$  and  $C_s$  are the concentrations, in mg per g, of the *Assay preparation* and the appropriate USP Reference Standard in the *Standard preparation*, respectively;  $r_u$  and  $r_s$  are the peak responses of the corresponding analyte obtained from the *Assay preparation* and the *Standard preparation*, respectively. The above results must be converted to the anhydrous basis.

A typical chromatogram for a commercial lot of Maltitol Solution, run according to the above method is shown below.



The specific intent of any proposed analytical method is the accurate quantitation of the primary component(s) in a Compendial Chemical or Preparation. For the purpose of validation any proposed method must, at a minimum, meet or exceed the criteria prescribed by the current USP method. What follows is a summary of the evaluation of a proposed method for the quantitation of the primary components in Maltitol Solution, maltitol and sorbitol. Said proposed analytical method carries the additional benefit of unification of the Compendial analysis of polyols in general.

Throughout the following summary references to "USP Conditions" implies the current USP Assay Procedure and references to "SPI Conditions" implies the Proposed Assay Procedure. Additionally, for the expressed purpose of an absolute comparison, all *Standard* and *Assay* preparations used for both methods were prepared on a weight/weight basis as directed in the proposed Analytical Procedure.

**ANALYTICAL PERFORMANCE CHARACTERISTICS****Accuracy**

In order to examine and compare the accuracy of the proposed method, five samples were prepared with known concentrations ranging from 5 to 15mg/g of USP Maltitol RS and 0.8 to 2.4 mg/g of USP Sorbitol RS. The five solutions were then chromatographed in triplicate according to both, the SPI and the USP methods. The results of this evaluation are summarized in the table below and demonstrate excellent recovery (98.0% - 100.0 %) for both analytes at all concentration levels with both methods.

**Accuracy: SPI conditions**

	<u>Known Conc. (mg/g)</u>	<u>Observed Conc. (mg/g)</u>	<u>% Recovery</u>
<b>Maltitol</b>	5.3	5.3	100
	7.7	7.6	98.7
	10.2	10.0	98.0
	12.9	12.7	98.4
	15.3	15.2	99.3
<b>Sorbitol</b>	0.8	0.8	100
	1.2	1.2	100
	1.6	1.6	100
	2.0	2.0	100
	2.4	2.4	100

**Accuracy: USP conditions**

	<u>Known Conc. (mg/g)</u>	<u>Observed Conc. (mg/g)</u>	<u>% Recovery</u>
<b>Maltitol</b>	5.3	5.3	100
	7.7	7.6	98.7
	10.2	10.0	98.1
	12.9	12.7	98.4
	15.3	15.1	98.7
<b>Sorbitol</b>	0.8	0.8	100
	1.2	1.2	100
	1.6	1.6	100
	2.0	2.0	100
	2.4	2.4	100

### Precision

The precision of a chromatographic method is the measure of the repeatability of analysis and to a greater extent is a measurement of the consistency of the entire chromatographic system. Therefore, in a situation where the chromatographic equipment remains unchanged and the proposed assay method requires only substitution of the column type and modifications to operating conditions such as temperature and flow rate, the comparison of precision between a reference method (USP) and a test method (SPI) would be largely influenced by the resolution and integration of the primary component peak(s).

To establish the precision of the SPI method three sample weights of a commercial lot of Maltitol Solution, Lot No. 80053, were prepared as directed under *Assay Preparation*. Five replicate injections per sample were chromatographed by both USP and SPI assay procedures. On two additional shifts fresh samples were prepared and chromatographed in an identical manner.

The data for both methods and both analytes was then evaluated by calculating the Mean, SD and % RSD for the replicates of the three samples within a shift, designated "within run", and for all replicates on all shifts, designated "total" precision. The replicate data for the SPI method is summarized in the table titled "Precision: SPI Conditions". The calculated "within run" and "total" precision values are presented in the same table.

A comparison of the "within run" and "total" precision between the USP and SPI methods is presented in a subsequent table titled "Comparison of Precision of Maltitol Solution Assays: USP and SPI". In all cases the random and systematic error as represented by the "within run" and "total" precision measurements are comparable for both assay methods.

**Precision: SPI conditions**

Sample #	Maltitol, % db			Sorbitol, % db		
	Shift #1	Shift #2	Shift #3	Shift #1	Shift #2	Shift #3
1	63.81	63.87	63.83	4.14	4.14	4.15
1	63.85	63.90	63.82	4.15	4.15	4.18
1	63.82	63.90	63.85	4.17	4.13	4.17
1	63.86	63.89	63.83	4.14	4.14	4.14
1	63.86	63.94	63.86	4.14	4.14	4.15
2	63.90	63.96	63.83	4.14	4.15	4.13
2	63.92	63.86	64.00	4.13	4.19	4.18
2	63.92	63.89	63.87	4.14	4.17	4.17
2	63.96	64.00	63.96	4.18	4.18	4.17
2	63.87	64.04	63.98	4.13	4.17	4.14
3	64.06	63.86	63.90	4.17	4.14	4.15
3	64.04	63.86	63.89	4.15	4.14	4.14
3	64.02	63.92	63.98	4.17	4.17	4.19
3	64.16	63.92	64.01	4.18	4.15	4.17
3	64.27	63.92	64.02	4.18	4.14	4.17

**Within Run Precision**

Avg	63.95	63.91	63.91	4.15	4.15	4.16
Std. Dev	0.132	0.051	0.074	0.019	0.018	0.018
%RSD	0.206	0.079	0.116	0.461	0.444	0.441

**Total Precision**

	Maltitol, % db	Sorbitol, %db
Average	63.93	4.16
Std. Dev.	0.092	0.0185
%RSD	0.144	0.444

**Precision (con't)****Comparison of Precision of Maltitol Solution Assays: USP vs. SPI****Maltitol, % DB**

		USP Conditions			SPI Conditions		
	Average	63.68	63.47	63.85	63.95	63.91	63.91
Within Run	Std. Dev.	0.094	0.060	0.244	0.132	0.051	0.074
	%RSD	0.147	0.094	0.383	0.206	0.079	0.116
	Average	63.67			63.93		
Total	Std. Dev.	0.217			0.092		
	%RSD	0.341			0.144		

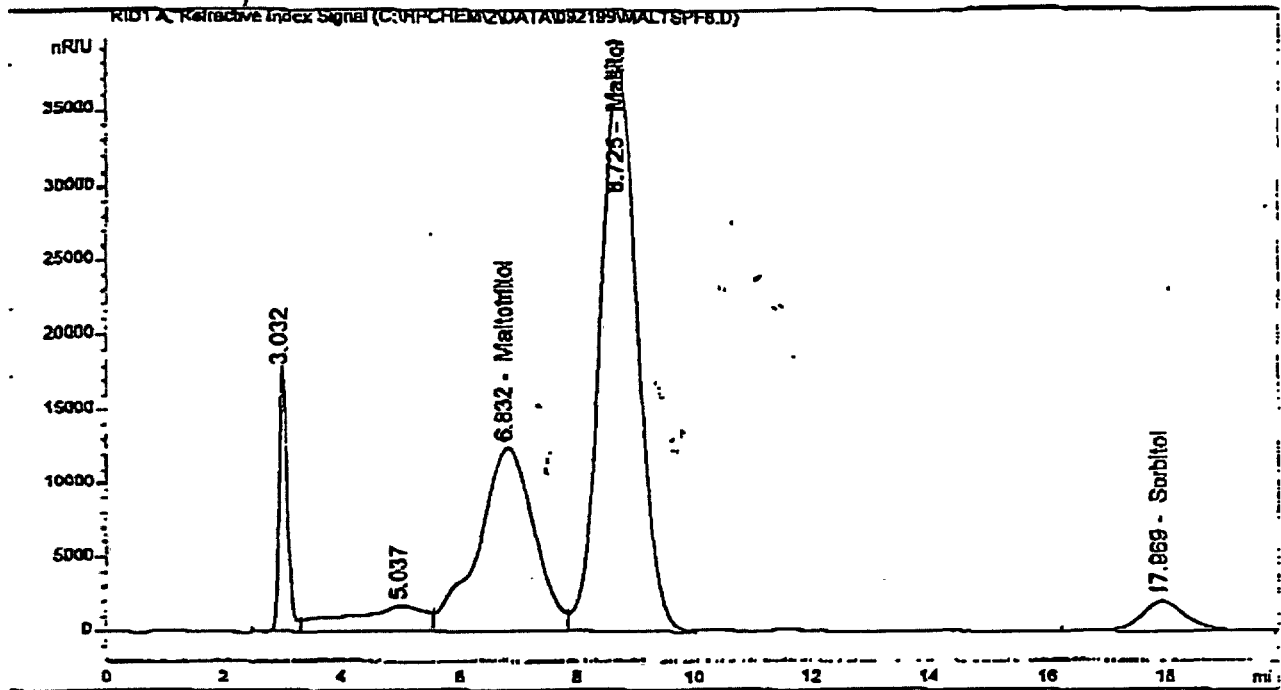
**Sorbitol, % DB**

		USP Conditions			SPI Conditions		
	Average	4.15	4.14	4.16	4.15	4.15	4.16
Within Run	Std. Dev.	0.010	0.006	0.016	0.019	0.018	0.018
	%RSD	0.236	0.150	0.385	0.461	0.444	0.441
	Average	4.15			4.16		
Total	Std. Dev.	0.015			0.018		
	%RSD	0.350			0.444		

**Specificity**

Maltotriitol and higher saccharides elute early in the analysis of Maltitol Solution with the maltotriitol being of primary concern because of its proximity to maltitol in the elution profile. Consequently, the specificity of the assay must address the impact of increasing concentrations of maltotriitol on the analytical recovery of a constant quantity of maltitol.

In order to examine the effects of maltotriitol on the resolution of maltitol, two preparations of a commercial lot of Maltitol Solution (Lot No. 80053) were spiked with 0.9 and 1.8 mg/g of pure maltotriitol. Each preparation was then chromatographed in triplicate without modification to the integration conditions established during calibration of the system. A representative chromatogram of Maltitol Solution containing an additional spike of 1.8 mg/g maltotriitol is presented below with a summary of the results of this evaluation being provided in the subsequent table. The consistency of the maltitol recoveries indicates that there is no impact on the quantification of maltitol in the presence of elevated concentrations of maltotriitol under the prescribed assay conditions.



typical production lot spiked with 1.8 mg/g maltitol (RT 6.832)

#### Specificity - SPI Conditions

Maltitol Spike Concentration, mg/g	Average Maltitol Assay, %db
0.0	63.93
0.9	63.95
1.8	63.94

Note: Assay values are the average of triplicate analysis. The %RSD's were all comparable to those in the precision section of this study.

#### Linearity

Since the intended use of the assay is to quantify both the maltitol and sorbitol in Maltitol Solution and, by definition Maltitol Solution may contain variable concentrations of both components it is critical that the prescribed assay remain linear over a range of concentrations. Furthermore, by definition, Maltitol Solution will contain proportions of the two primary components such that sorbitol is present at significantly lower concentrations than maltitol. This ratio of components thus necessitates the evaluation of linearity over two different ranges.

To examine the linearity of the assay for the two components, five preparations containing known concentrations of approximately 5 to 15 mg/g of USP Maltitol RS and 0.8 to 2.4 mg/g of USP Sorbitol RS were chromatographed in triplicate. The repeatability of the analysis was established by performing a second series of evaluations with fresh standards.

The data obtained from this analysis was evaluated by plotting the Observed Response versus the Known concentrations. Regression analysis was then performed using only the prescribed *Standard* concentration and the concentrations representing the *Standard*  $\pm$  25%, represented by samples 2, 3 and 4 in the data tables for both maltitol and sorbitol. The regression lines were then used to predict the Theoretical Recovery of all data points and the linearity established by calculating the difference between the Known and Theoretical concentrations. Treatment of the data in this manner permits a more critical evaluation at the extremes while the correlation coefficients of the regressed data establish the integrity of the fit within the working range.



A summary of the data from these evaluations is presented in the table titled "Maltitol Assay Linearity". The Linearity plots and the regression analysis comparing the SPI assay and the current USP assay are presented in the subsequent graphs.

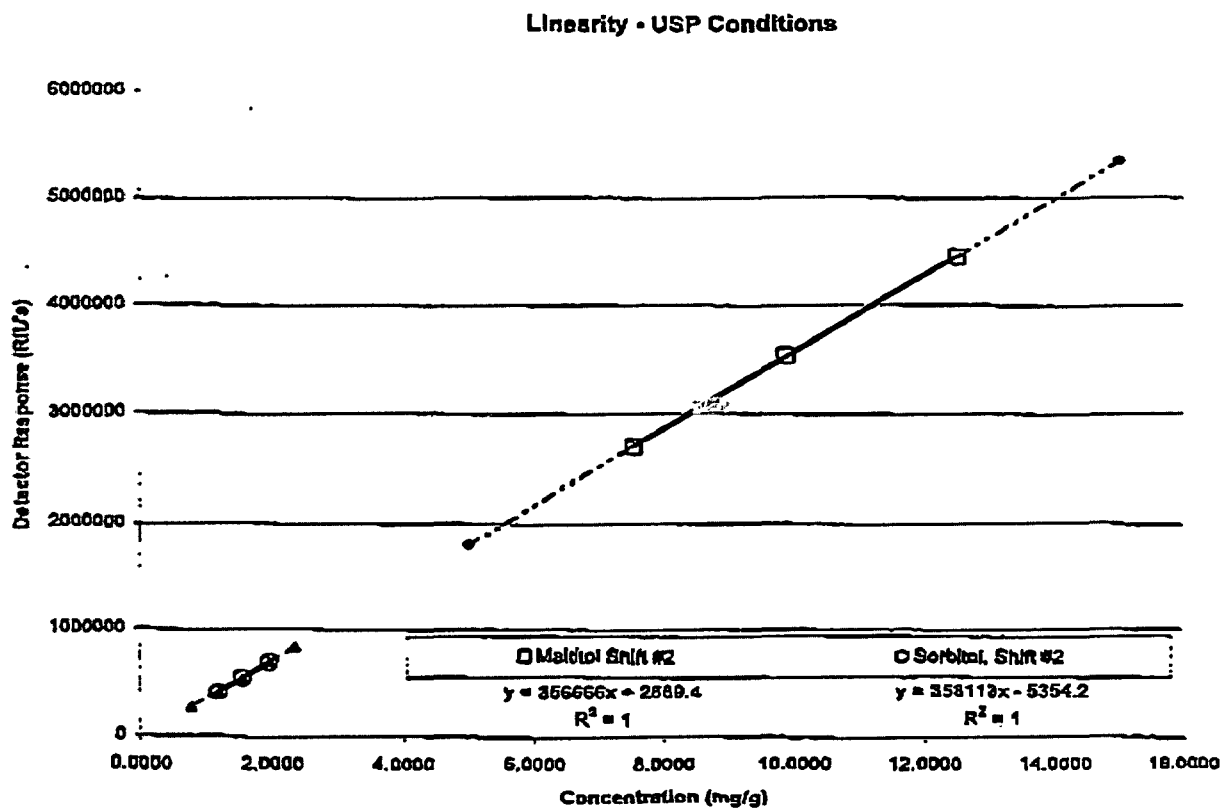
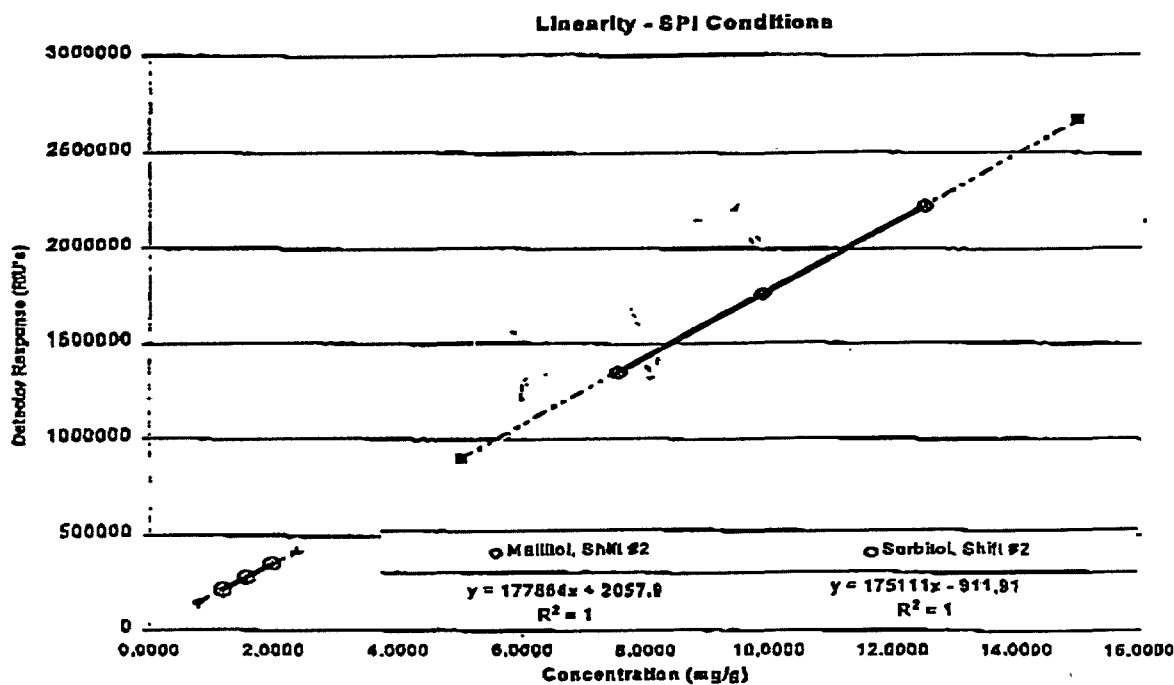
In all cases, the correlation coefficients for the regressed data for both analytes shows that there is virtually no deviation between the Known and Theoretical concentrations within the working range. Additionally, the minimal deviation of the Theoretical Recoveries predicted at the extremes indicate that both assay procedures are linear beyond the range examined in this study. This is substantiated by the magnitude of the slope of the regressed line for the USP assay which is double that of the SPI assay. Although this is a direct result of the difference in injection volume between the two methods it indicates a linear detector response over a much greater concentration range.

**Maltitol Assay Linearity (units of concentration, mg/g)**

SPI Conditions						USP Conditions					
	Sample Number	Avg. Rsp.	Known Conc.	Obs. Conc.	Δ Conc		Sample Number	Avg. Rsp.	Known Conc.	Obs. Conc.	Δ Conc
Shift #1	1	961867	5.3	5.4	0.1	Shift #1	1	1924070	5.3	5.4	0.1
	2	1369503	7.7	7.7	0.0		2	2750107	7.7	7.7	0.0
	3	1809147	10.2	10.2	0.0		3	3634613	10.2	10.2	0.0
	4	2291273	12.9	12.7	0.2		4	4586063	12.8	12.8	0.0
	5	2743243	15.3	15.2	0.1		5	5478723	15.3	15.1	0.2
Shift #2	1	899481	5.0	5.0	0.0	Shift #2	1	1802557	5.0	5.0	0.0
	2	1344423	7.5	7.5	0.0		2	2690830	7.5	7.5	0.0
	3	1759973	9.9	9.9	0.0		3	3534340	9.9	9.9	0.0
	4	2225947	12.5	12.5	0.0		4	4458703	12.5	12.5	0.0
	5	2679703	15.0	15.1	-0.1		5	5344417	15.0	15.0	0.0

**Sorbitol Assay Linearity (units of concentration, mg/g)**

SPI Conditions						USP Conditions					
	Sample Number	Avg. Rsp.	Known Conc.	Obs. Conc.	Δ Conc		Sample Number	Avg. Rsp.	Known Conc.	Obs. Conc.	Δ Conc
Shift #1	1	142181	0.8	0.8	0.0	Shift #1	1	283429	0.8	0.8	0.0
	2	208363	1.2	1.2	0.0		2	417191	1.2	1.2	0.0
	3	276177	1.6	1.6	0.0		3	554135	1.6	1.6	0.0
	4	350581	2.0	2.0	0.0		4	702198	2.0	2.0	0.0
	5	417623	2.4	2.4	0.0		5	834956	2.4	2.4	0.0
Shift #2	1	137687	0.8	0.8	0.0	Shift #2	1	275501	0.8	0.8	0.0
	2	207698	1.2	1.2	0.0		2	414540	1.2	1.2	0.0
	3	271829	1.6	1.6	0.0		3	545902	1.6	1.6	0.0
	4	344263	2.0	2.0	0.0		4	690089	2.0	2.0	0.0
	5	413649	2.4	2.4	0.0		5	828111	2.4	2.4	0.0



### Ruggedness

Evaluation of ruggedness is intended to establish that a method will provide equivalent results on different systems under normal test conditions. In as much as the SPI method involves only a column substitution with moderate alterations to operating temperature and flow rate, an abbreviated examination of ruggedness was performed. For purposes of this validation the accuracy and "total" precision of the method, which are most likely to be impacted by changes of the type described, were evaluated on a second chromatographic system. Additionally, five lots of commercial Maltitol Solution were analyzed on two different systems to evaluate the performance of the method in routine operation.

With respect to accuracy, five solutions were prepared with known concentrations ranging from 5 to 15mg/g of USP Maltitol RS and 0.8 to 2.4 mg/g of USP Sorbitol RS. The solutions were then chromatographed in triplicate on each of two separate chromatographic systems according to the SPI method. The results of this evaluation are summarized in the table titled "Ruggedness - Accuracy". The data shows no difference between systems and demonstrates excellent recovery for both analytes at all concentration levels.

**Ruggedness - Accuracy, (units of concentration, mg/g)**

Analyte	Known Conc.	Instrument #1		Instrument #2	
		Obs. Conc.	% Recovery	Obs. Conc.	% Recovery
Maltitol	5.0	5.0	100	5.0	100
	7.5	7.5	100	7.5	100
	9.9	9.9	100	9.9	100
	12.5	12.5	100	12.5	100
	15.0	15.0	100	15.0	100
Sorbitol	0.8	0.8	100	0.8	100
	1.2	1.2	100	1.2	100
	1.6	1.6	100	1.6	100
	2.0	2.0	100	2.0	100
	2.4	2.4	100	2.4	100

"Total" precision was evaluated by analyzing the same nine samples prepared in the original Precision study over a two day period. The results, presented in the table titled "Ruggedness - Precision", are quite comparable to the "total" precision obtained in the original study as well as with that of the USP method.

**Ruggedness - Precision**

	Instrument #1, %db		Instrument #2, %db		Rel. Variance, %	
	Maltitol	Sorbitol	Maltitol	Sorbitol	Maltitol	Sorbitol
Average	63.93	4.16	63.87	4.19	0.09	0.72
Std. Dev.	0.09	0.07	0.26	0.02		
%RSD	0.14	0.44	0.40	0.49		

The results of routine analysis of five commercial lots of Maltitol Solution on two different chromatographic systems are summarized in the table titled "Ruggedness - Production Lot Analysis". Comparison of the assay values between the two systems shows agreement well within acceptable tolerances for both analytes.

**Ruggedness - Production Lot Analysis**

Lot No.	Instrument #1, Assay %db		Instrument #2, Assay %db		Δ Assay %db	
	Maltitol	Sorbitol	Maltitol	Sorbitol	Maltitol	Sorbitol
80529	64.71	4.61	64.76	4.59	0.05	-0.02
80532	65.37	3.78	65.38	3.76	0.01	-0.02
90287	64.05	4.48	63.94	4.45	-0.11	-0.03
80528	64.63	5.06	64.21	5.03	-0.42	-0.03
90286	62.74	5.57	62.95	5.60	0.21	0.03

### Robustness

Evaluations of robustness are intended to determine how small but deliberate changes to method conditions will impact the analytical results. To determine these effects samples of Production Lot No. 80053 were prepared as directed under *Assay Preparation* and chromatographed in duplicate at column temperatures ranging from 40 °C to 80 °C and flow rates from 0.4 mL/min to 0.9 mL/min. System calibration and chromatographic integration parameters were established at the original operating conditions and were held constant without further optimization throughout the investigation.

The data in the following table demonstrates that alteration or drift in the instrumental conditions may appear as a moderate variance in the analytical results for maltitol. The increase in recovery of maltitol is a result of decreased resolution between the maltotriitol and maltitol especially at temperatures below 50 °C and above 60 °C or at flow rates of less than 0.4 mL/min or greater than 0.7 mL/min. This does not, however, preclude the use of alternate temperatures or flow rates within a limited range but indicates that recalibration and optimization of the integration parameters may be necessary.

Conditions Temp (°C)	Flow rate (mL/min)	Average Percent Assay, db	
		Maltitol	Sorbitol
40	0.5	65.97	4.11
40	0.7	68.64	4.12
45	0.5	65.05	4.16
45	0.7	65.10	4.11
50	0.5	64.50	4.15
50	0.9	65.53	4.17
55	0.5	64.37	4.17
55	0.7	64.52	4.15
60	0.4	65.67	4.09
60	0.5	63.93	4.16
60	0.7	64.08	4.13
80	0.5	65.26	4.23
80	0.7	64.57	4.19

System Suitability

System suitability, as defined under the *Chromatographic system*, is measured as the Relative Standard Deviation of the tailing factor for maltitol and sorbitol calculated from replicate injections of the *Standard Preparation*. Throughout the course of this validation study, the *Standard Preparation* was analyzed at regular intervals. The tailing factor was monitored as a system suitability check to insure continuity in the data collection. The data presented in the following table shows the tailing factors for both analytes are close to 1.0 and the RSD's are less than 1%.

Date	USP Tailing Factor	
	Maltitol	Sorbitol
8/17/99	0.998	0.971
8/17/99	0.985	0.974
8/17/99	0.990	0.973
8/17/99	0.992	0.978
8/17/99	0.984	0.980
8/17/99	0.988	0.979
8/17/99	0.990	0.976
8/17/99	0.985	0.977
8/19/99	0.994	0.984
8/19/99	0.994	0.987
8/19/99	0.991	0.993
8/19/99	0.989	0.990
8/19/99	0.995	0.989
8/19/99	0.995	0.991
8/20/99	0.994	0.992
8/20/99	0.992	0.988
8/20/99	0.995	0.997
8/20/99	0.995	0.997
8/20/99	0.993	0.994
Avg.	0.991	0.985
Std. Dev.	0.004	0.008
% RSD	0.391	0.859

**Comparison of Methods:**

Although not a specific requirement of Compendial Methods Validations any study of this type should include a comparison between the two methods as an evaluation of performance under normal operation. For this purpose, ten production lots were assayed in accordance with both the USP and SPI procedures.

The comparative data is presented in the table titled "Comparison of Methods – Maltitol Solutions". Each assay result is the average of four analysis (duplicate analysis of two assay preparations) for each production lot. The difference between assay results for the two methods is less than 1% relative in all cases for both analytes and is, therefore, not considered to be significant.

**Comparison of Methods – Maltitol Solution**

Lot Number	SPI Conditions		USP Conditions	
	Maltitol, %db	Sorbitol, %db	Maltitol, %db	Sorbitol, %db
90288	63.85	4.74	63.86	4.70
80531	64.71	3.75	64.74	3.77
80527	63.47	5.65	63.52	5.64
80530	64.77	4.07	64.73	4.07
80529	64.71	4.61	64.51	4.59
80532	65.37	3.78	65.50	3.80
80053	64.09	4.17	63.81	4.15
90287	64.05	4.48	63.90	4.46
80528	64.63	5.06	64.82	5.08
90286	62.74	5.57	63.17	5.59

PR



# Appendix E

APPENDIX E

000189

**APPENDIX E**  
**EXHIBIT S 1.0**  
**HYSTAR 6075**

000190





# Consumer Product Testing

Company Incorporated

Bldg No. 2-15B  
1275 Bloomfield Avenue  
Fairfield, New Jersey 07006

(201) 575-7688  
(201) 575-7689

## FINAL REPORT

**CLIENT:**

Lonza Inc.  
22-10 Route 208  
Fair Lawn, New Jersey 07410

**ATTENTION:**

Peter J. Schaeufele  
Assistant Corporate Technical Director

**TEST:**

Acute Oral LD<sub>50</sub> in Rats

**TEST  
ARTICLE:**

Compound W-59-5 - Hystar 6075

**EXPERIMENT  
REFERENCE NO.:**

B1354-5

~~Steven Nitka~~  
Laboratory Director

~~Allen L. Palanker~~  
President

Date January 19, 1982  
SN/tmc

This report is submitted for the exclusive use of the person, partnership, or corporation to whom it is addressed and neither the report nor the name of these Laboratories nor that of any member of its staff may be used in connection with the advertising or sale of any product or process without written authorization.

000191

This report details:

an acute oral LD<sub>50</sub> study in albino rats,

performed at the behest of:

Lonza Inc.  
22-10 Route 208  
Fair Lawn, New Jersey 07410

The test article(s), supplied by:

Lonza Inc.

received on:

October 7, 1981

and identified as:

Compound W-59-S

was used as indicated in the Final Report Summaries.

Study Interval: October 21, 1981 to November 30, 1981

000192

(201) 575-7688  
(201) 575-7689



# Consumer Product Testing

Company Incorporated

Bldg No. 2-15B  
1275 Bloomfield Avenue • Fairfield, New Jersey 07006

## QUALITY ASSURANCE UNIT SUMMARY

Study No.: 81354-5

The objective of the Quality Assurance Unit (QAU) is to monitor the conduct and reporting of nonclinical laboratory studies as set forth in the Good Laboratory Practice regulations (21 CFR 58). The QAU maintains copies of study protocols and standard operating procedures and has inspected this study on the date(s) listed below. Studies lasting six months or more are inspected every three months; and studies lasting less than six months are inspected at time intervals to assure the integrity of the study. The findings of these inspections have been reported to management and Study Director. All materials and data pertinent to this study will be stored in the Archives Facility.

Date(s) of inspections:	October 14, 1981	November 11, 1981
	October 21, 1981	November 18, 1981
	October 28, 1981	January 21, 1982
	November 4, 1981	

Professional personnel involved:	Steven Nitka, B.S.	- Laboratory Director (Study Director)
	Sheila Johnson, B.S.	- Technician
	Ellen Lally, B.A.	- Technician
	Barbara Schultz, B.S.	- Technician
	Pam Emerson	- Technician Assistant
	Irene Komrovsky	- Technician Assistant
	Nancy Somer	- Technician Assistant
	Kirk Puryear	- Technician Assistant
	Deborah A. Worman	- Office Manager

The following has been assured by signing below that this study has been performed in accordance with standard operating procedures and the Good Laboratory Practice regulations.

~~Janet K. Johnson~~  
Director of Quality Assurance

000193

(201) 575-7688  
(201) 575-7689



# Consumer Product Testing

Company Incorporated

Bldg No. 2-15B  
1275 Bloomfield Avenue ● Fairfield, New Jersey 07006

## Final Report Summary

DATE: January 19, 1982  
CLIENT: Lonza Inc.  
STUDY NO.: 81354-5  
REFERENCE: P.J. Schaeufele  
TEST ARTICLE: Compound W-59-5

### Acute Oral LD<sub>50</sub> in Rats

Method: Albino rats, 218-280 g, sexes distributed as indicated below, were dosed singly at range finding levels and in groups of ten (5M:5F) per test level. Each animal received one (1) oral dose of the test article. Animals were observed for pharmacologic activity and drug toxicity at 1, 3, 6, and 24 hours after treatment, and daily thereafter for a total of 14 days. Animals sacrificed at the end of the 14 day observation period, as well as non-survivors, were subjected to gross necropsy, with all findings noted. The test article was used as received (Sp.g. = 1.38).

LD<sub>50</sub> > 40g/kg\*

	<u>Dose Level</u> (g/kg)	<u>Sex</u>	<u>No. Dead/No. Dosed</u> (M:F)	<u>Mortality</u> (%)
Range				
Finding:	1.00	1M	0/1	0
	10.00	1M	0/1	0
	20.00	1F	0/1	0
	40.00	1F	0/1	0
Test Dose				
Levels:	40.00	5M:5F	1/5:1/5	20

\*Maximum dosage.

000194

This test was designed to determine the acute oral LD<sub>50</sub> of the test article in rats. The methods described by Hagan<sup>1</sup> served as a guide.

Wistar-strain albino rats were used for this test. Animals were obtained from a suitably licensed dealer, in equal numbers of each sex and approximately 6 to 9 weeks of age. Upon receipt, the animals were carefully checked for respiratory difficulty, ocular or nasal lacrimation, dehydration, diarrhea, and general thriftiness.

The animals were acclimated for at least 5 days prior to test initiation. They were housed in galvanized cages with indirect bedding, in a temperature controlled room with a 12 hour light/dark cycle. Diet consisted of a growth and maintenance ration from a commercial producer and water ad libitum.

Prior to test initiation, the test article's mass to volume relationship (specific gravity) was determined to facilitate volumetric dosing.

An initial phase of the test, a dosage level range finding, was performed to determine a possible range for the LD<sub>50</sub>. One rat was dosed at each of several dose levels, with a wide spread between successive levels. The lowest dose level at which mortality occurred served as the guide for choosing the first of several graded dose levels used for the LD<sub>50</sub> calculation.

Twenty-four (24) hours prior to dosing, all rats were reexamined for general thriftiness as described above. A group of rats, sexes equally distributed, and of sufficient weight to assure a fasted bodyweight between 200 and 300 grams, was labelled and set aside.

The following day, after approximately 18 hours of fasting, each rat was weighed and marked with an ear clip. Individual doses, calculated on the basis of bodyweight and the dose level being administered, were given using a stainless steel intragastric feeding needle of sufficient bore to allow even passage of the test article in its dosing form. Rats were then returned to their cages, where food and water were available ad libitum. Each cage was uniquely labelled with respect to job number, test article, dose level, sex, animal number(s), and date of dosing.

The animals were observed for signs of pharmacologic activity and drug toxicity at 1, 3, 6, and 24 hours post-dosage. Observations were made at least once daily thereafter for a total of 14 days.

Animals sacrificed at the end of the 14 day observation period, as well as non-survivors, were weighed and subjected to complete gross necropsy, with all findings noted.

The oral LD<sub>50</sub>, including 95% confidence limits, was calculated where possible using the method of Litchfield and Wilcoxon.<sup>2</sup>

<sup>1</sup>E.C. Hagan, "Acute Toxicity", Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics (The Association of Food and Drug Officials of the United States, 1975), pp. 17-25.

<sup>2</sup>J.T. Litchfield and F. Wilcoxon, "A Simplified Method of Evaluating Dose-Effect Experiments", Journal of Pharmacology and Experimental Therapeutics, 96 (1949), pp. 97-177.

000195

Acute Oral LD50 in Rats

Individual results are presented in Tables 1 through 2.

Summaries of all results are found preceding the text.

000196

Table 1

Acute Oral Toxicity

Compound W-59-5

Lonza Inc.  
81354-5  
Page 7

Dose Level: Range Finding (g/kg)																			
Animal Number and Sex	Bodyweight (grams)	Hours:			Days:														Bodyweight (grams)
		1	3	6	24	2	3	4	5	6	7	8	9	10	11	12	13	14	
1 M- 1.00	280	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	434
2 M-10.00	266	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	396
3 F-20.00	242	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	302
4 F-40.00	236	SD	SD	SD	N	N	N	N	N	N	N	N	N	N	N	N	N	N	324

Raw Data Page 8406

N = Normal  
 D = Depression  
 SD = Slight Depression  
 XD = Severe Depression  
 H = Hyperactivity  
 + = Animal Death

1 Hair moist and matted  
 2 Hair matted and unkempt  
 3 Probable middle ear infection  
 4 Diarrhea  
 5 Mucoïd diarrhea  
 6 Appears dehydrated  
 7 Convulsions  
 8 Muscle tremors

Comments: Animal #1-#4: No gross changes observed.

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Table 2  
Acute Oral Toxicity

Compound N-59-5

Lonza Inc.  
81354-5  
Page 8

Dose Level: 40.00 g/kg

Animal Number and Sex	Bodyweight (grams)	Hours:				Days:														Bodyweight (grams)
		1	3	6	24	2	3	4	5	6	7	8	9	10	11	12	13	14		
1 M	222	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	310	
2 M	224	SD	SD	SD	SD	N	N	N	N	N	N	N	N	N	N	N	N	N	278	
3 M	230	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	354	
4 M	236	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	354	
5 M a	260	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	354	
6 F	232	SD	XD	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	226	
7 F	226	N	SD	SD <sup>5</sup>	+	-	-	-	-	-	-	-	-	-	-	-	-	-	200	
8 F	218	N <sup>5</sup>	N <sup>5</sup>	N <sup>5</sup>	N	N	N	N	N	N	N	N	N	N	N	N	N	N	270	
9 F	230	N <sup>5</sup>	N <sup>5</sup>	N <sup>5</sup>	N	N	N	N	N	N	N	N	N	N	N	N	N	N	300	
10 F	220	N	N	N <sup>5</sup>	N	N	N	N	N	N	N	N	N	N	N	N	N	N	276	

Raw Data Page 8426 and 8466

N = Normal  
D = Depression  
SD = Slight Depression  
XD = Severe Depression  
H = Hyperactivity  
+ = Animal Death

1 Hair moist and matted  
2 Hair matted and unkempt  
3 Probable middle ear infection  
4 Diarrhea  
5 Mucoid diarrhea  
6 Appears dehydrated  
7 Convulsions  
8 Muscle tremors

Comments: Animals #1-#4, #5a, #8-#10: No gross changes observed.  
#6: Gastrointestinal mucosa severely reddened.  
#7: No gross changes observed.  
Original animal #5 replaced: Fibrous tissue encasing heart and lungs.

000198

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**APPENDIX E**  
**EXHIBIT S 1.2**

000199



# Consumer Product Testing Co.

## FINAL REPORT


**CLIENT:** Grain Processing Corporation  
1600 Oregon Street  
Muscatine, Iowa 52761


**ATTENTION:** Rani M. Thomas  
Director of Quality and  
Regulatory Affairs

**TEST:** Acute Oral LD<sub>50</sub> in Rats

**TEST ARTICLE:** Experimental M180

**EXPERIMENT  
REFERENCE NUMBER:** T00-0040

~~~~  
Kathleen Alworth, B.A.  
Director of Quality Assurance

~~~~ 3/31/00  
Steven Nitka  
Laboratory Director  
Vice President

000200

This report is submitted for the exclusive use of the person, partnership, or corporation to whom it is addressed, and neither the report nor the name of these Laboratories nor any member of its staff, may be used in connection with the advertising or sale of any product or process without written authorization.



EST. 1975

# Consumer Product Testing Co.

## QUALITY ASSURANCE UNIT STATEMENT

**Study No.:** T00-0040

The objective of the Quality Assurance Unit (QAU) is to monitor the conduct and reporting of nonclinical laboratory studies. These studies have been performed with strict adherence to the Good Laboratory Practice Act (21 CFR 58) and in accordance to standard operating procedures and applicable standard protocols. The study is listed on this facility's Master Schedule. The QAU maintains copies of study protocols and standard operating procedures and has inspected this study on the date(s) listed below. The findings of these inspections have been reported to management and the Study Director. All materials and data pertinent to this study will be stored in the Archive Facility at 70 New Dutch Lane, Fairfield, New Jersey, 07004, unless specified otherwise, in writing by the Sponsor.

### **Dates of biophase/data inspection/Report to Management:**

March 9, 2000	March 10, 2000	March 13, 2000
March 14, 2000	March 20, 2000	March 21, 2000
March 22, 2000	March 23, 2000	March 27, 2000
March 28, 2000	March 31, 2000	

### **Professional personnel involved:**

Steven Nitka, B.S.	- Vice President
	Laboratory Director
	(Study Director)
Lillian Deniza, B.S.	- Laboratory Supervisor
Melissa Pandorf, B.S.	- Technician
Essam Eldeib, Ph.D.	- Quality Assurance Associate

The representative signature of the Quality Assurance Unit on the front page signifies that this study has been performed in accordance with standard operating procedures and applicable study protocols.

000201



EST. 1975

# Consumer Product Testing Co.

## Final Report Summary

**CLIENT:** Grain Processing Corporation

**STUDY NO.:** T00-0040

**REFERENCE:** R.M Thomas

**TEST ARTICLE:** Experimental M180

**TEST ARTICLE RECEIPT DATE:** February 29, 2000

**EXPERIMENTAL INTERVAL:** March 10, 2000 to March 28, 2000

## Acute Oral LD<sub>50</sub> in Rats

**Method:** Albino rats, 200 - 230 g, sexes as indicated below, were dosed singly at range finding levels and in a test level group of ten (5M:5F). Each animal received a single oral dose of the test article. Animals were observed for pharmacological activity and drug toxicity 1, 3, 6, and 24 hours after treatment, and daily thereafter for a total of 14 days. All animals survived the observation period and were then euthanized and subjected to a gross necropsy with all findings noted. The test article was used as a 25% suspension in corn oil.

<b>Results:</b>	<b>Dose Level</b> (g/kg)	<b>Sex</b>	<b>No. Dead/No. Dosed</b> (M:F)	<b>Mortality</b> (%)
Range				
Finding:	1.00	1M	0/1	0
	5.00	1F	0/1	0
	10.00	1F	0/1	0
Test Dose				
Level:	10.00	5M:5F	0/5:0/5	0

**Conclusion:** LD<sub>50</sub> > 10 g/kg (Ten (10) grams per kilogram is the maximum feasible dosage at the concentration of 25%).

000202

### Acute Oral LD<sub>50</sub> in Rats

This test was designed to determine the acute oral LD<sub>50</sub> of the test article in rats. The methods described by Hagan<sup>1</sup> served as a guide.

Wistar-strain, albino rats were used for this test. Animals were obtained from Ace Animals in Boyertown, Pennsylvania, in equal numbers of each sex and approximately six to nine (6 to 9) weeks of age. Upon receipt, the animals were carefully checked for respiratory difficulty, ocular or nasal lacrimation, dehydration, diarrhea, and general condition.

The animals were acclimated for at least seven (7) days prior to test initiation. They were housed in stainless steel cages with indirect bedding, in a room with a 12 hour light/dark cycle. The room temperature was controlled, to provide for the health and comfort of the animals with an approximate range of 65° to 75° F. The humidity was also monitored. Diet consisted of Lab Diet Certified Rodent Diet #5002, as well as water, *ad libitum*.

Prior to test initiation, the test article was suspended in corn oil at 25%. Fresh suspensions were made on each dosing day.

An initial phase of the test, a dosage level range finding with initial dosages as chosen by the sponsor, was performed to determine a possible range for the LD<sub>50</sub>. One (1) rat was dosed at each of several dose levels, with a wide spread between successive levels. The dose levels served as the guide for choosing the test dose level used for the LD<sub>50</sub> calculation.

Twenty-four (24) hours prior to dosing, all rats were reexamined for general condition as described above. A group of rats, sexes equally distributed, and of sufficient weight to assure a fasted body weight between 200 and 300 grams, was labeled and set aside.

The following day, after approximately 18 hours of fasting, each rat was weighed and marked with an ear clip. Individual doses, calculated on the basis of body weight and the dose level being administered, were given using a stainless steel intragastric feeding needle of sufficient bore to allow even passage of the test article in its dosing form. Rats were then returned to their cages, where food and water were available *ad libitum*. Each cage was uniquely labeled with respect to job number, test article, dose level, sex, animal number(s), and date of dosing.

The animals were observed for signs of pharmacological activity and drug toxicity at 1, 3, 6, and 24 hours post-dosage. Observations were made at least once daily thereafter for a total of 14 days. All animals survived the observation period and were then euthanized and subjected to a gross necropsy with all findings noted. Sacrificing was accomplished via carbon dioxide asphyxiation.

<sup>1</sup>E.C. Hagan, "Acute Toxicity", *Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics*, (The Association of Food and Drug Officials of the United States, 1959), pp. 17 - 25.

**Acute Oral LD<sub>50</sub> in Rats**

The individual test results are presented in Tables 1 and 2.

Summaries of all results are found preceding the text.

**Table 1**  
**Acute Oral Toxicity**  
**Experimental M180**

Dose Level RF @ 1,5 + 10 g/kg																			
Animal Number and Sex	Bodyweight (grams)	Hours:				Days:													
		1	3	6	24	2	3	4	5	6	7	8	9	10	11	12	13	14	Bodyweight (grams)
1 M	227	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	330
2 F	200	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	235
3 F	201	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	243

Raw Data Page 17287

N = Normal

D = Depression

SD = Slight Depression

XD = Severe Depression

H = Hyperactivity

+ = Animal Death

<sup>1</sup>Hair moist and matted

<sup>2</sup>Hair matted and unkempt

<sup>3</sup>Probable middle ear infection

<sup>4</sup>Diarrhea

<sup>5</sup>Mucoid diarrhea

<sup>6</sup>Appears dehydrated

<sup>7</sup>Convulsions

<sup>8</sup>Muscle tremors

Necropsy comments: Animals #1-#3: No gross changes observed.

000205

Table 2

Acute Oral Toxicity

Experimental M180

Dose Level 10 g/kg

Animal Number and Sex	Bodyweight (grams)	Hours:				Days:														Bodyweight (grams)
		1	3	6	24	2	3	4	5	6	7	8	9	10	11	12	13	14		
1 M	220	N	N	N	N <sup>1</sup>	N	N	N	N	N	N	N	N	N	N	N	N	N	338	
2 M	222	N	N	N	N <sup>1</sup>	N	N	N	N	N	N	N	N	N	N	N	N	N	336	
3 M	213	N	N	N	N <sup>1</sup>	N <sup>2</sup>	N	N	N	N	N	N	N	N	N	N	N	N	326	
4 M	230	N	N <sup>5</sup>	N <sup>5</sup>	N <sup>1</sup>	N <sup>2</sup>	N	N	N	N	N	N	N	N	N	N	N	N	356	
5 M	224	N	N	N	N <sup>1</sup>	N <sup>2</sup>	N	N	N	N	N	N	N	N	N	N	N	N	348	
6 F	218	N	N	N	N <sup>1</sup>	N	N	N	N	N	N	N	N	N	N	N	N	N	276	
7 F	216	N	N	N	N <sup>1</sup>	N	N	N	N	N	N	N	N	N	N	N	N	N	278	
8 F	224	N <sup>5</sup>	N <sup>5</sup>	N <sup>5</sup>	N <sup>1</sup>	N <sup>2</sup>	N	N	N	N	N	N	N	N	N	N	N	N	268	
9 F	209	N <sup>5</sup>	N <sup>5</sup>	N <sup>5</sup>	N <sup>1</sup>	N <sup>2</sup>	N	N	N	N	N	N	N	N	N	N	N	N	262	
10 F	217	N	N	N	N <sup>1</sup>	N <sup>2</sup>	N	N	N	N	N	N	N	N	N	N	N	N	276	

Raw Data Page 17289

N = Normal  
D = Depression  
SD = Slight Depression  
XD = Severe Depression  
H = Hyperactivity  
+ = Animal Death

<sup>1</sup>Hair moist and matted  
<sup>2</sup>Hair matted and unkempt  
<sup>3</sup>Probable middle ear infection  
<sup>4</sup>Diarrhea  
<sup>5</sup>Mucoid diarrhea  
<sup>6</sup>Appears dehydrated  
<sup>7</sup>Convulsions  
<sup>8</sup>Muscle tremors

000206

Necropsy comments: Animals #1-#10: No gross changes observed.  
Index to FDA Response to NRDC's FOIA Request No. 2013-8042 for GRN-59



**APPENDIX E****EXHIBIT S 2.0****ROQUETTE GRAS AFFIRMATION PETITION #84G-0003 SECTION IV  
INFORMATION TO ESTABLISH SAFETY AND FUNCTIONALITY IN FOOD**

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iv. Information to Establish Safety  
and Functionality in Food

SAFETY\*

SUMMARY

In June 1983, a panel of experts composed of past members of the Select Committee on GRAS Substances of the Federation of American Societies for Experimental Biology, after a review of the reports and documents contained in Sections ii and iv (but not including the functionality data) of this petition, concluded (Report E-51) that:

There is no evidence in the available information on LYCASIN 80/55 that demonstrates a hazard to the public when it is used at levels that are now current and in the manner now practiced or that might reasonably be expected in the future.

Approximately 96 percent of Lycasin 80/55 is ultimately broken down in the digestive tract into sorbitol and glucose. One half of the remaining four percent, in the form of maltitol, is excreted in the feces, the rest enters the blood stream and is excreted intact in the urine.

The LD<sub>50</sub> of Lycasin 80/55 is equal to or higher than sorbitol. In subchronic studies, Lycasin 80/55 produces no significant toxic effects which cannot be accounted for by the

000208

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\* Reports E-1 through E-51, pertaining to the safety of hydrogenated glucose sirip, are presented in Appendix E.

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presence of sorbitol. Lycasin 80/55 is nonmutagenic, nonclastogenic, and produces no significant toxic effects on reproduction.

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day

Lycasin 80/55 may produce a laxative effect at an intake level of approximately 100g per day. To reach this threshold level, an individual would have to consume considerable quantities of products containing Lycasin 80/55. For example, the 100g per day intake threshold is equivalent to 4-5 rolls of hard candy mints, 50 jelly beans, 111 sticks of chewing gum or 180 miniature marshmallows (these figures are based on data provided in Appendix C).

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#### DIGESTION, ABSORPTION, DISTRIBUTION, AND EXCRETION

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Within the first two hours after oral administration, Lycasin 80/55 is broken down into two GRAS substances, sorbitol and glucose, and maltitol. Within 7 hours, 95 percent of the maltitol is broken down into sorbitol and glucose. Of the remaining maltitol one-half is excreted in the feces, one-fourth is excreted in the urine and one-fourth remains in the blood stream. After 12 hours, the blood levels of maltitol are practically zero and there is no accumulation in the other tissues or organs of the body.

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#### ACUTE TOXICITY

The LD<sub>50</sub> of Lycasin 80/55 is equal to or greater than that of sorbitol and glucose.

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SUBCHRONIC TOXICITY

Lycasin 80/55, when administered orally to rats and dogs in amounts of 5 to 15 grams per kilogram of body weight per day for 90 days, produces no toxicologically meaningful effects which cannot be accounted for by the presence of sorbitol.

The possible treatment-related effects are aggregates in the renal pelvis of some rats, diarrhea in most dogs and minimal ectasia in the renule tubules of some dogs.

GENETIC TOXICITY/CARCINOGENICITY

Lycasin 80/55 is nonmutagenic and nonclastogenic in short term in vivo and in vitro studies. A similar material containing at least 75 percent maltitol, when administered to rats over a 78-week period, produces no carcinogenic effects.

REPRODUCTION TOXICITY

Lycasin 80/55, when administered to rats over a three-generation period, produces no significant effects on reproduction.

DIGESTIVE AND BIOLOGICAL TOLERANCE IN MAN

Lycasin 80/55, at doses up to 180 grams per day, produces no significant variation in the clinical chemical, hematological, or urinary profile, with the exception of serum glucose and insulin peaks which are less than 50 percent of those produced by an equivalent amount of glucose.

The only clinical effects are flatulence and diarrhea, which can be attributed to the presence of free and bound sorbital. The mean laxative threshold in adult males is

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approximately 180 grams per day, while in females the threshold is approximately 100 grams per day. In children, the threshold is approximately 60 grams per day, about half that of adults.

POTENTIAL HUMAN EXPOSURE

The potential average daily consumption of Lycasin 80/55 by eaters, those who consume candies and chewing gums, is estimated to be 0.5 to 1.1 grams per day, with a 90th percentile (high eaters) consumption level at 1.1 to 2.6 grams per day.

NOTE: LYCASIN 80/55, LYCASIN 80/33 AND LYCASIN 65/63

Although this petition seeks approval of the product, Lycasin 80/55, chemical and biological data are also presented on the prototype products, Lycasin 80/33 and Lycasin 65/63. These data are presented solely for the purpose of providing secondary verification of the Lycasin 80/55 studies, especially in those instances where the original data on Lycasin 80/55 studies were unavailable for post study audit. A comparison of the chemical composition of these products is provided in report E-50.

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SUMMARY OF THE  
DIGESTION, ABSORPTION, DISTRIBUTION AND  
EXCRETION OF LYCASIN 80/55

SUMMARYLYCASIN<sup>®</sup> 80/55

DP1	= sorbitol	: 7 %				S	Free Sorbitol	FS
DP2	= maltitol	: 52,5 %				G <sub>1-4</sub> - S	Free Maltitol	FM
DP3	= maltotriitol	: 18 %		G <sub>1-4</sub>		G <sub>1-4</sub> - S		
DP4	=	: 1 %		G <sub>1-4</sub> -G <sub>1-4</sub>		G <sub>1-4</sub> - S		
DP5	=	: 2 %		G <sub>1-4</sub> -G <sub>1-4</sub> -G <sub>1-4</sub>		G <sub>1-4</sub> - S		
(DP6 to DP20)	= polyitols	: 19,5 %		G <sub>1-4</sub> -G <sub>1-4</sub> -G <sub>1-4</sub> -G <sub>1-4</sub> -G <sub>1-4</sub>		G <sub>1-4</sub> - S		
							Total Sorbitol	TS
							43,6 %	
							Total Maltitol	TM
							(TS-FS) × 2 =	73,2 %

Over 96 percent of Lycasin 80/55 is broken down by the mammalian digestive system into the GRAS substances, glucose and sorbitol; the remaining 4 percent is in the form of maltitol. One half of the maltitol is excreted in the feces and the majority of the remainder is excreted in the urine.

Within the first two hours after oral administration of Lycasin 80/55, virtually all of the glucose to glucose bonds are broken down in the digestive system, producing a resulting mixture of glucose, sorbitol, and maltitol.

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- . Within 7 hours, 95 percent of the total maltitol, (free maltitol plus the maltitol moiety of higher saccharide alcohols) is broken down into glucose and sorbitol. Of the remaining 5 percent of maltitol (equivalent to 3.66 percent of the Lycasin 80/55 ingested), 2 percent is found in the digestive tube and fecal contents, less than 1 percent is found in the plasma, and approximately 1 percent is excreted in the urine.
- . There is no accumulation of maltitol in the plasma, liver, kidneys, or spleen of rats fed 13.5 g/kg/day of Lycasin 80/55 for 10 days. This is true whether measurements are made 12 hours or 10 days after cessation of dosing.

#### DIGESTION

- . 50 to 85 percent of the glucose molecules contained in Lycasin 80/55 are released through hydrolysis when the product is incubated in the presence of amylo-glucosidase for 4 to 6 hours (Report E-1, Cf generally Report E-40).
- . When Lycasin 80/55 is incubated in the presence of either  $\alpha$ -glucosidase or homogenates of rat small intestine, it releases approximately 50 percent of the glucose molecules as compared to an equivalent amount of its precursor high maltose corn syrup (Report E-2).

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- . When Lycasin 80/55 is incubated in the presence of either rat intestinal mucosa (pylorus to rectum) or human intestinal mucosa (jejunum), it releases both glucose and sorbitol.
  - The hydrolysis of glucose to glucose bonds proceeds rapidly.
  - The hydrolysis of glucose to sorbitol bonds (maltitol) proceeds more slowly (Report E-3).
- . The glucose to glucose bonds of Lycasin 80/55 are rapidly hydrolyzed within the first 1 1/2 hours after oral administration to rats. Within 7 hours 95 percent of the glucose to sorbitol bonds (maltitol) are hydrolyzed (Report E-4).
- . Lycasin 80/55 and high maltitol syrup are metabolized by man to approximately the same extent as mixtures of glucose and sorbitol in equivalent ratios (Report E-41).
- . 80 percent of the glucose moieties in Lycasin 65/63 are readily split off by the digestive enzymes of the rat, the maltitol moieties, in contrast, are split very slowly (Report E-42).
- . Maltitol is split into glucose and sorbitol molecules by the mucosal disaccharidases of rat, rabbit and man. This splitting occurs more slowly than with sucrose; the rate of hydrolysis may be compared to that of lactose. Approximately 70% of the ingested

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maltitol is hydrolyzed by the time it has reached the distal portion of the rats intestines (Report E-44).

- . Maltitol is hydrolyzed in the stomach into glucose and sorbitol. Maltitol is also subjected to microbial fermentation in the cecum producing volatile fatty acids (Report E-45).
- . A comparison between germ-free and conventional rats indicate that approximately 12 percent of ingested maltitol is metabolized by the intestinal microflora of rats (Report E-41).

#### ABSORPTION

- . Approximately 9, 5, and 2 percent respectively of Maltitol-U-<sup>14</sup>C is transported across jejunal, ileal and duodenal everted sacs of rat intestine when incubated for 60 minutes at 37°C (Report E-14).
- . Approximately 1 percent of the potential maltitol in 200 to 600 mg of Lycasin 80/55 ingested by rats is absorbed in tact into the bloodstream as determined by urinary excretion. Maltitol is detectable in the plasma only sporadically during the 7 hours of observation and always at very low levels (0.2 mg/ml or less) (Report E-4).
- . Approximately 1 percent of the potential sorbitol in 200 to 600 mg of Lycasin 80/55 ingested by rats is absorbed into the bloodstream as determined by urinary excretion. Sorbitol is detectable in the plasma

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at levels of 0.15 to 0.90 mg/ml during the 7 hour period of observation. There appears to be no dose/response relationship (Report E-4).

- . Less than 1 percent of orally administered  $^{14}\text{C}$ -maltitol appears in the blood stream of mice within the first 6 hours after oral administration. Chromatographic analysis indicates that the radiolabeled substances in the blood are principally  $^{14}\text{C}$ -glucose and  $^{14}\text{C}$ -sorbitol (Report E-5).
- . Lycasin 80/55 produces a serum glucose peak within 60 minutes after oral administration in rats. This peak is approximately three-fourths that produced by its precursor, high maltose corn syrup (peak at 30 minutes) (Report E-6).
- . Lycasin 80/55 produces serum glucose and insulin peaks within 30 minutes after oral administration in man. These peaks are approximately one-half to one-third of an equivalent amount of glucose (Report E-7).
- . Lycasin 80/55 produces serum glucose and insulin peaks within 30 to 60 minutes after oral administration in normal and diabetic humans. These peaks are approximately 50 percent of those produced by an equivalent volume of glucose and approximately 50 to 90 percent of those produced by sucrose (Report E-8).
- . Lycasin 65/63 produces a serum glucose peak within 30 minutes after oral administration in man. This peak

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is essentially equal to that produced by an equivalent amount of dextrose (Reports E-9 and E-10).

- Lycasin 65/63 produces a rise in the blood glucose level of man equal to that produced by an equivalent amount of sucrose (Report E-43).
- The glucose units of the maltitol molecules and moieties of Lycasin 65/63 largely escape being absorbed in the small intestines of rats. Sorbitol, whether free or bound, is absorbed very slowly (Report E-42).

#### DISTRIBUTION AND EXCRETION

- Lycasin 80/55 does not produce a significant increase in liver glycogen when administered orally to rats maintained on a carbohydrate-deficient diet. Its precursor, high maltose corn syrup, produces a significant increase (16 percent) under the same conditions (Report E-11).
- When administered orally to rats for 28 days, Lycasin 80/55 does not produce significant differences in liver glycogen, carcass fat, ash, moisture, or protein content as determined by proximate analysis when compared to an equivalent amount of its precursor, high maltose corn syrup, or sucrose (Report E-12).
- There is no accumulation (detection limit 0.1 mg per organ or mg per ml of plasma) of maltitol in the plasma, liver, kidneys, or spleen of rats fed 13.5

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g/kg/day of Lycasin 80/55 for 10 days. The maximum rate of excretion of maltitol in the urine over the same period is less than 0.2 percent (15.8 mg/kg/day). There is also no significant difference between the free sorbitol levels in the liver, plasma, and spleen of control and Lycasin-fed animals, nor are there detectable levels in the kidneys of either group (Report E-13).

- . Seven hours after oral administration of 600 mg of Lycasin 80/55 to rats, 55 percent of the material (based on total sorbitol recovered) is found in the digestive tube and fecal contents and less than one percent is found in each of the plasma and urine. The remainder of the material, in the form of glucose and sorbitol, has presumably entered the body's metabolic pathways (Report E-4).
- . Approximately 30 percent of the radiolabeled material appears in the feces of rats 6 hours after oral administration of maltitol-U-<sup>14</sup>C. An additional 3 percent appears in the total respiratory CO<sub>2</sub>. The majority of the remainder of the activity is found in the digestive tube contents (Report E-5).
- . 83 percent of the recovered radiolabeled material appears in the caecal, large intestine and fecal contents of rats 24 hours after oral administration of maltitol-U-<sup>14</sup>C. An additional 3 percent appears in

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the stomach and small intestinal contents. Six percent appears in the urine and 1.6 percent appears in the total respiratory CO<sub>2</sub>. Less than 0.3 percent appears in each of the blood, liver, muscle, brain, and kidney organs. Less than 0.1 percent appears in each of the heart, lung, spleen, pancreas, stomach, large intestines, caecum, testes, and adipose tissue organs (Report E-14).

- . 88 percent of the recovered radiolabeled material appears in the urine of rats 24 hours after intravenous administration of maltitol-U-<sup>14</sup>C. An additional 3.5 percent appears in the total respiratory CO<sub>2</sub>. Less than 0.3 percent appears in each of the blood, liver, muscle, heart, and kidney organs (Report E-14 and Report E-41).

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SUMMARY OF  
ACUTE TOXICITY OF LYCASIN 80/55

SUMMARY

The acute, LD<sub>50</sub>, toxicity of Lycasin 80/55 is comparable to that of sorbitol and glucose.

REPORTS

- The LD<sub>50</sub> (14 days) of Lycasin 80/55 in mice is:

Grams per kilogram of body weight

Route	Sex	
	Male	Female
Oral	> 24.0	> 24.0
Intravenous	> 6.4	> 8.2
Intraperitoneal	> 10.6	> 12.4

(Report E-15)

- The LD<sub>50</sub> (14 days) of Lycasin 80/55 in rats is:

Grams per kilogram of body weight

Route	Sex	
	Male	Female
Oral	> 24.4	> 24.4
Intraperitoneal	> 13.0	> 13.0

(Report E-16)

- The oral LD<sub>50</sub> of sorbitol in male and female mice is 23.2 and 25.7 grams per kilogram of body weight respectively. The oral LD<sub>50</sub> in male and female rats is 17.5 and 15.9 grams per kilogram of body weight respectively (cf. Report E-17). The intraperitoneal LD<sub>50</sub> in mice is 15 grams per kilogram of body weight (NIOSH, RTECS). 000220

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- The oral LD<sub>50</sub> of glucose in rats, dogs and rabbits is 25.8, 8 and 20 grams per kilogram of body weight respectively. The intravenous LD<sub>50</sub> in rabbits is 12 grams per kilogram of body weight. The intraperitoneal LD<sub>50</sub> in mice is 18 grams per kilogram of body weight. (NIOSH, RTECS).

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000221

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SUMMARY OF THE  
SUBCHRONIC TOXICITY OF LYCASIN 80/55

SUMMARY

Lycasin 80/55, when administered to rats and dogs in the amount of 5 to 15 grams per kilograms of body weight for 90 days, produces no toxicologically meaningful effects which cannot be accounted for by the presence of sorbitol. (See generally reports E-17, E-46, E-47, E-48 and E-49) The possible treatment-related effects are:

- Aggregates in the renal pelvis of some rats
- Diarrhea in most dogs
- Minimal ectasia in the renal tubules of some dogs.

REPORTS

Clinical chemical and hematological examinations and urinalysis on rats fed diets containing 10, 15, and 20 percent of Lycasin 80/55 for 13 weeks revealed no treatment-related effects when compared to an equivalent diet of 20 percent sucrose. Lycasin 80/55 also produces no significant histopathological effects, with the possible exception of aggregates in the renal pelvis (Report E-18). If this effect is treatment-related, it is probably due to the sorbitol content of Lycasin 80/55 (cf. Report E-17).

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- When fed to rats as 20 percent of the diet for 90 days, Lycasin 80/55 produces no toxicologically meaningful effects on body weight, mortality, clinical manifestation, food consumption, hematological profile, clinical chemical profile, urine profile, relative organ weights, or histopathological appearance when compared to an equivalent amount of sorbitol diet (Report E-19).
- When fed to rats as 2, 5, and 15 percent of the diet for 90 days, Lycasin 65/63 produces no toxicologically meaningful effects on body weight gain, mortality, clinical manifestations, food consumption, hematological profile, clinical chemical profile, urine profile, or histopathological appearance when compared to rats maintained on a standard laboratory diet (Report E-20).
- When orally administered to beagle dogs at a level of 5 grams per kilogram of body weight per day for 90 days, Lycasin 80/55 produces no toxicologically meaningful effects on mortality, clinical manifestations, body weight gain, opthamological profile, hematological profile, myelographic profile, clinical chemical profile, PAH clearance, urine profile, or histopathological appearance when compared to untreated animals with the following exceptions:
  - Diarrhea

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- Slight increase in plasma triglycerides of females
- Minimal ectasia in the renal tubules.

These observations must be considered as being physiologic, in relation to the high amount of saccharide alcohols provided by the treatment (Report E-21).

- . Lycasin 65/63, when fed to beagle dogs as 2, 5, and 15 percent of the diet for 90 days, produces no toxicologically meaningful effects on mortality, body weight gain, food consumption, clinical manifestations, hematological profile, clinical chemical profile, urine profile, organ weights, or histopathological appearance when compared to untreated animals (Report E-22).

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SUMMARY OF  
GENETIC TOXICITY OF LYCASIN 80/55

SUMMARY

- . Lycasin 80/55, as demonstrated in an extensive series of 8 in vivo and in vitro genetic toxicology studies, is nonmutagenic and nonclastogenic. From this it is concluded that Lycasin 80/55 is unlikely to have a carcinogenic potential.
- . A similar material containing at least 75 percent maltitol, when administered to rats over a 78 week period, produces no carcinogenic effects.

REPORTS

- . Lycasin 80/55, at concentrations ranging from 0.003 to 40.0 mg/ml of incubation mixture, does not produce any significant increases in cellular toxicity or reversion to histidine prototrophy in the S. typhimurium strains TA 1535, TA 1537, TA 1538, TA 98, or TA 100 either in the presence or absence of s9 metabolic activators (Report E-23).
- . Lycasin 80/55, at concentrations ranging from 4.0 to 280.0 mg/ml of incubation mixture, does not produce any significant increases in reversion to histidine prototrophy in the S. typhimurium strains TA 1535, TA 1537, TA 98, and TA 100 either in the presence or absence of s9 metabolic activator (Report E-24).

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- Lycasin 80/55, when fed to rats at doses ranging from 2.0 to 15.0 grams per kilogram of body weight for 14 days, does not produce urinary metabolites which are active in inducing reversions to histidine prototrophy in S. typhimurium strains, TA 1535, TA 1537, TA 1538, TA 98, and TA 100 either in the presence or absence of s9 metabolic activation (Report E-25).
- Lycasin 80/55, at concentrations of 0.003 to 30.0 mg/ml of incubation mixture, does not produce any significant increase in the inductive capacity of E. coli K-12, strain GY5027 with Lambaphage (2 pgsa) either in the presence or absence of s9 metabolic activation (Report E-26).
- Lycasin 80/55, at doses of 5.0 to 25.0 grams per kilogram body weight, does not significantly increase the number of micronuclei carrying polychromatophilic erythrocytes in mice (Report E-27).
- Lycasin 80/55, at concentrations ranging from 10.0 to 1000.0 ug/ml of incubation mixture, does not significantly increase the frequency of Type III morphologically transformed foci or cytotoxicity in C3H/10T 1/2 (Clone 8) cells either in the presence or absence of s9 metabolic activators (Report E-28).
- Lycasin 80/55, at concentrations ranging from 49.0 to 4900.0 ug/ml of incubation mixture, does not

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significantly increase the frequency of chromosomal aberrations in Chinese Hamster Ovary cells, either in the presence or absence of s9 metabolic activators (Report E-29).

- . Lycasin 80/55, at concentrations ranging from 27.0 to 1000.0 mg/ml of incubation mixture, does not significantly increase the frequency of mouse lymphoma mutations either in the presence or absence of s9 metabolic activators (Report E-30).
- . It is unlikely that Lycasin 80/55, which has been demonstrated to be nonmutagenic and nonclastogenic in an extensive battery of genetic toxicology procedures, has the potential to produce carcinogenic effects (Report E-31).
- . A material consisting of at least 75 percent matitol, when administered to rats at doses of 1.5 to 5.5 grams per kilogram of body weight for 78 weeks, produces no carcinogenic effects (Report E-32).

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SUMMARY OF THE TOXIC EFFECTS  
OF LYCASIN 80/55 ON REPRODUCTION

SUMMARY

Lycasin 80/55, when administered over three generations, produces no significant effects on reproduction.

BASIS

- . Lycasin 80/55, when administered to rats over three generations at dose levels of 13.0 to 17.5 grams per kilogram of body weight per day, produces no significant regularly recurring effects on:
  - Male or female fertility
  - The gestation period
  - The occurrence of pup malformations
  - The number of live and stillborn births
  - Postnatal survival
  - Adult survival
  - Adult hematological parameters
  - Postmortem macroscopic observations
  - Gastrointestinal tract.
- . Lycasin 80/55 does produce the following effects:
  - Transitory decrease in pre-weanling growth rate
  - Reduced adult male kidney weight
  - Increased adult male fecal weight.

(Report E-33)

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SUMMARY OF THE DIGESTIVE AND  
BIOLOGICAL TOLERANCE OF MAN OF LYCASIN 80/55

SUMMARY

- . Lycasin 80/55, at the dose levels tested, 30 to 180 grams per day, produces no significant variations in the clinical chemical, hematological or urinary profile of man with the exception of glucose and insulin peaks which are less than 50 percent of those produced by equivalent amounts of glucose, and 50 to 90 percent of those produced by sucrose.
- . The only significant clinical effects are flatulence and diarrhea, which can be accounted for by the presence of free and bound sorbital. (See generally reports E-17, E-46, E-47, E-48 and E-49).
- . The mean laxative threshold in adult males is approximately 180 grams per day, while in females the threshold is approximately 100 grams per day. This difference is possibly due to the fact that females digest and absorb Lycasin 80/55 more slowly than males. (Cf Report E-4). With a higher saccharide alcohol content in the lower digestive tract, the osmotic gradient would produce watery stools.
- . The mean laxative threshold in children appears to be around 60 grams per day, approximately half that of

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adults. Therefore Lycasin 80/55 appears to exert its laxative effect on a grams per kilogram body weight basis.

BASIS

- In adult males the mean laxative threshold for Lycasin 80/55, after repetitive daily consumption, appears to be greater than 180 grams per day, while in adult females it appears to be at or above 100 grams per day. Lycasin 80/55, when administered in doses of 30 to 180 grams per day for 5 to 120 days, produces no significant effects on the clinical chemical profile or urine volume of normal or diabetic subjects other than mild transitory hyperglycemia (Report E-34).
- The mean laxative threshold for Lycasin 80/55 in men and women is approximately 125 grams per day (Report E-41).
- Lycasin 80/33, when administered to adult males and females in doses of 45 to 90 grams per day for 30 to 90 days, produces no significant laxative effect. At the doses tested, Lycasin 80/33 produces no significant effect on clinical chemical or hematological profile of normal or diabetic subjects other than a mild transitory hyperglycemia (Report E-35).

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- . Lycasin 80/55, when administered in single doses of 50 to 100 grams, produces peaks in serum glucose and insulin which are approximately one-half to one-third, respectively, of those produced by an equivalent amount of glucose. Subjects given 100 grams occasionally experience abdominal pain and flatulence which disappear overnight (Report E-7).
- . Lycasin 80/55, when administered to adult males and females in the form of 48 to 58 grams of hard boiled candies per day for six days, produces the same number of symptomless days as a similar amount of sucrose candies; however, the number of gastrointestinal symptoms reported per subject day for Lycasin 80/55 are approximately two to five times higher than those for sucrose (Report E-36).
- . Lycasin 80/55, when administered to adult males and females in doses of 70 grams per day for 28 days, produces diarrhea on less than 7 percent and gas pains on less than 20 percent of the subject days. Over this same period Lycasin 80/55 does not produce any significant changes in the clinical chemical, hematological, or urinary profile (Report E-37).
- . After repeated daily consumption of Lycasin 80/55, the human digestive system appears to adapt, resulting in gradual disappearance of diarrhea and flatulence over 4-5 days (Report E-41).

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- . Lycasin 80/55, when administered to children, ages 3 to 14, in doses of 9 to 60 grams per day over a one-hour period, produces gas pains in 14 percent of the subject days at the 20 gram level, 20 percent at the 30 gram level, 50 percent at the 40 gram level, 75 percent at the 60 gram level, while loose stools are produced in 8 percent of the subject days at the 30 to 40 gram level and 50 percent at the 60 gram level (Report E-38).
- . Lycasin 80/55 produces serum glucose and insulin peaks within 30 to 60 minutes after oral administration in normal and diabetic subjects. These peaks are approximately 50 percent of those produced by an equivalent amount of glucose and approximately 50 to 90 percent of those produced by sucrose (Report E-8).

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SUMMARY OF THE  
LYCASIN 80/55 POTENTIAL  
HUMAN EXPOSURE ASSESSMENT

SUMMARY

The potential average daily consumption of Lycasin 80/55 by "eaters", those individuals who consume candies, confections and chewing gum, is estimated to be 0.5 to 1.1 grams per day, with a 90th percentile (high eaters) consumption level at 1.1 to 2.6 grams per day.

BASIS

- . The potential average daily per capita consumption of Lycasin 80/55 is estimated to be 45 to 107 milligrams per day (Report E-39).
- . The potential average daily consumption of Lycasin 80/55 by "eaters", is estimated to be 0.5 to 1.1 grams per day, with a 90th percentile consumption level at 1.1 to 2.6 grams per day (Report E-39).
- . The potential single day (peak) average daily consumption of Lycasin 80/55 by eaters is estimated to be 1.2 to 2.7 grams per day, with a 90th percentile consumption level at 2.4 to 5.9 grams per day (Report E-39).

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## FUNCTIONALITY\*

The functionality of Lycasin 80/55 in candy, chewing gum and confections is due to the following technical effects:

- . Sweetness
- . Hygroscopicity or aw depressing capacity (Humectant)
- . Binding capacity
- . Anticrystallizing capacity
- . Absence of reducing capacity.

Lycasin 80/55 has approximately 75 percent the sweetness of sucrose (Report F-1). Because the sweetness is less than sugar, the acid flavors currently used in confections (e.g., citric acid, lactic acid, etc.) are more easily tasted. It is, therefore, possible to reduce, by about 25 to 30 percent, the quantities of acid normally used in candies (Report F-2). In addition, Lycasin 80/55 contains no sulphur dioxide (SO<sub>2</sub>), a substance which sometimes may cause deterioration of flavors.

Lycasin 80/55 gives finished products with a lower E.R.H. (aw) than that of standard confectionery containing sugar (Reports F-1 and F-2). It is therefore hygroscopic and a humectant, and has very good bacteriological stability. In the case of boiled confectionery, use of Lycasin 80/55 yields hygroscopic sweets which will not recrystallize on the surface if water is absorbed (Report F-2).

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\* Reports F-1 through F-11, pertaining to the functionality of hydrogenated glucose sirip, are presented in Appendix F.

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Lycasin 80/55 has an average molecular weight nearly twice that of sucrose; 630 versus 342 (Report F-1). The longer the molecules, the higher the cohesive effect. Its relatively high viscosity, 2000 cps at 20°C, enables it to be easily worked. In finished products, it behaves like a high dextrose equivalent glucose syrup (Report F-2). This technical effect is extensively used in confection production; for example, chewing gum, soft coatings and fillings (Report F-1).

Lycasin 80/55 never crystallizes even at low temperatures or when its concentration is increased. Like a glucose syrup, it prevents crystallization of other components which may be present in the formulation such as sorbitol, mannitol and xylitol. This also explains Lycasin's numerous applications in confectionery, since sorbitol, mannitol, xylitol, etc. have a marked tendency to recrystallize, which can therefore bring about an unwanted change in texture of the finished products during storage (Reports F-1, F-2 and F-3).

Lycasin 80/55 contains practically no reducing sugars; less than 0.2 percent. It is therefore very stable, with little tendency to brown on heating. For the same reason, it does not react with other components of confection formulations; i.e., absence of Maillard reaction in particular (Report F-2).

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Sample Recipes

Developmental recipes for sugarless confectionaries containing Lycasin 80/55 are presented in Report F-4.

- . Soft gums
- . Chewy sweets
- . Marshmallows
- . Gelatin jellies
- . Soft jellies
- . Coated chewing gum
- . Chewing gum
- . Wine gums
- . Hardboiled candies

(Cf Report F-5)

Additional information on the use of Lycasin 80/55 in chewing gums is provided in Reports F-6 and F-7. Information on the use of Lycasin 80/55 in sugarless soft coatings for jelly beans is provided in Report F-8.

Lycasin 80/55 hydrogenated glucose sirip remains essentially unchanged during the manufacture of finished candies and confections. High temperature processing and/or addition of citric acid at the end of the manufacturing process does promote a small amount of  $\alpha$ -1-4 bond hydrolysis releasing small quantities of glucose and sorbitol. (Report F-4).

The stability of Lycasin 80/55, when stored for two years under typical warehouse conditions was good (Report F-10), as was the stability of an 18% Lycasin 80/55 solution when stored for 10 days at room temperature (Report F-11).

**APPENDIX E**  
**EXHIBIT 3.0**  
**HYSTAR LAXATIVE RANGE FINDING STUDY**  
**DATA SUMMARY**

HYSTAR LAXATIVE RANGE FINDING STUDIES

## DOSE LEVELS AT 100% ACTIVE

HIGH A = 150GM/PER DAY OR 2.1GM/KG BODY WEIGHT  
MEDIUM B = 100GM/PER DAY OR 1.4GM/KG BODY WEIGHT  
LOW C = 50GM/PER DAY OR 0.7GM/KG BODY WEIGHT

PRODUCTS TESTED

G-37-1 = HYSTAR 7000  
G-37-2 = HYSTAR 6075  
G-37-3 = HYSTAR HM-75  
G-37-4 = SORBITOL CONTROL  
G-37-5 = SUGAR/CORN SYRUP CONTROL


000238  




TABLE 1  
EXCUSED FROM STUDY BECAUSE OF SEVERE G.I. TRACT  
DISTURBANCES

GROUP I.D.	DAY 1	DAY 2	DAY 3	DAY 4
DAY 5 TOTALS				
<u>G-37-1 (HYSTAR 7000)</u>				
NO. OF SUBJS. A (HIGH)	4			4
B (MED)	3	1		4
C (LOW)	2	1	1	4
<u>G-37-2 (HYSTAR 6075)</u>				
NO. OF SUBJS. A (HIGH)	2	2		4
B (MED)		1		1
C (LOW)				0
<u>G-37-3 (HYSTAR HM-75)</u>				
NO. OF SUBJS. A (HIGH)	1	3		4
B (MED)			1	3
C (LOW)			1	1
<u>G-37-4 (SORBITOL CONTROL)</u>				
NO. OF SUBJS. A (HIGH)				0
B (MED)				0
C (LOW)		1		1
<u>G-37-5 (SUGAR/CORN SYRUP)</u>				
NO. OF SUBJS. A (HIGH)				0
B (MED)				0
C (LOW)				0

000239

**APPENDIX E**

**EXHIBIT 3.1**

**A CLINICAL EVALUATION OF THE LAXATIVE POTENTIAL OF A  
SERIES OF HYDROGENATED STARCH HYDROLYSATES AND  
RELATED PRODUCTS**

000240

## **A CLINICAL EVALUATION OF THE LAXATIVE POTENTIAL OF A SERIES OF HYDROGENATED STARCH HYDROLYSATES AND RELATED PRODUCTS**

### **BACKGROUND AND OBJECTIVE OF THE STUDY**

Sorbitol is widely used as a sweetening agent in hard and soft candies, chewing gum and other confectionary products. When ingested in large quantities, many people may experience gastrointestinal difficulties such as flatulence and diarrhea.

An addition to the uses mentioned above, certain investigators believe that sorbitol does not produce the same rise in blood sugars when ingested, as does sucrose or other sugars, and accordingly, may be incorporated in confectionaries for people on restricted sugar diets.

Three new products (hydrogenated starch hydrolysates) intended as substitutes for sorbitol, were tested to determine their laxative potential and other gastrointestinal side effects as well as to what degree they caused a rise in blood sugars, in normal human volunteers.

### **SUMMARY**

Hystar 7000, Hystar HM-75 and Hystar 6075 were tested at three- (3) dosage levels in groups of twelve (12) individuals. The control product was sugar/corn syrup. Sorbitol, another control, was tested in four (4) subjects at one (1) dosage level (0.7 g/kg) as it is known that sorbitol in a single dose of 50 grams will induce diarrhea in a majority of subjects. Doses were administered daily for a period of five (5) consecutive days. The criteria for discontinuance from the study (laxation "end-point") were an incidence of severe diarrhea, for one day or diarrhea and severe flatulence for 2 consecutive days.

Hystar 6075 caused the least gastrointestinal effect at all dosage levels, five (5) subjects (four (4) on the high dose and one (1) on the mid dose) being discontinued because of diarrhea and flatulence. Hystar 7000 caused severe G.I. disturbances in all subjects on all dosage levels, while Hystar HM-75 caused these side effects in 8 of 12 subjects – all four (4) on the high dose, three (3) on the mid dose and one (1) on the low dose (see table I).

No one was discontinued because of gastrointestinal problems on the sugar/corn syrup control group, at any of the three dose levels. One (1) of four (4) subjects on sorbitol (0.7 g/kg) was discontinued because of G.I. symptoms.

Blood sugar levels in all test subjects were similar for all test products at 30 minutes post-dosing and at all the other sampling except in the sugar/corn syrup control group. It appears that blood sugars were raised at all doses and at all time intervals for the first five (5) days, but were normal on the Day 7 determinations. Of three Hystar compounds, Hystar 6075 appeared to cause higher blood sugars at 30 and 60 minutes post-dosing.

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**TESTING FACILITY**

Essex Testing Clinic, Inc., 799 Bloomfield Avenue, Verona, NJ 07044.

Principal Investigator: Harold Schwartz, Ph.D., President.

Panel No: 84-085

Sponsor: L5

**TEST MATERIALS**

The test articles were supplied by the Study Sponsor, Lonza, Inc., Fairlawn, NJ 07410. They were received on November 2, 1984 and were identified as follows:

Date Rec'd	Quantity Rec'd	Code	Description
11/2/84	2 gallons	G-37-1	50% wt/wt sol'n of Hystar 7000
11/2/84	2 gallons	G-37-2	50% wt/wt sol'n of Hystar 6075
11/2/84	2 gallons	G-37-4	50% wt/wt sol'n of Sorbitol Control
11/2/84	2 gallons	G-37-5	50% wt/wt sol'n of Sugar/Corn Syrup control

Additional test materials were received as follows:

11/9/84	1 gallon	G-37-3	50% wt/wt sol'n of Hystar HM-75
11/9/84	1.5 gallons	G-37-5	50% wt/wt sol'n of Sugar/Corn Syrup Control
11/15/84	1 gallon	G-37-3	50% wt/wt sol'n of Hystar HM-75
11/15/84	1 gallon	G-37-2	50% wt/wt sol'n of Hystar 6075

**STUDY DESIGN**

Twelve subjects (6 males and 6 females) between the ages of 26 and 55 with "normal" bowel habits formed the test group for each compound except sorbitol, where four (4) subjects formed the group. Each group (except sorbitol) was divided into three (3) sub-groups of four (4) subjects (2 males and 2 females) in a randomized fashion. Ingestion of the test and control products was on a daily basis for a period of five (5) days. Sub-group A received a "high" dose of 2.1 grams per kilogram of body weight each day; Sub-group B received a "middle" dose of 1.4 g/kg each day; while Sub-group C was given a "low" dose of 0.7 g/kg each day.

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The four- (4) subjects (2 male and 2 female) assigned to the sorbitol control received 0.7 g/kg each day. All dosage calculations were based on a "high dose" of 150 g/day, "middle dose" of 100 g/day and a "low dose" of 50 g/day for both men and women. The test materials were prepared by the Study Sponsor as 50% "active" concentrations (wt/wt), therefore each subject was dosed at 2 x body weight so that the test article "active" dose was as indicated above.

All subjects admitted to the study reported daily to Essex Testing Clinic, Inc., 799 Bloomfield Avenue, Verina, NJ. On day one (Monday) they were randomly assigned to a dosage group and, after reading and signing an Informed Consent form (see Appendix I) Received instructions and a diary to record their daily diets and whether or not any G.I. distress was experienced. A blood glucose determination was made at that time (initial or baseline) and again 30, 60 and 180 minutes after dosing. An Ames Glucomete<sup>R</sup> (Model 5580) and Ames Dextrostix<sup>R</sup> Reagent Strips were used for whole blood glucose determinations from fingertip puncture. Additional blood glucose determination were made each succeeding day (through day 5) and again on day seven (7).

Each subject was given a dose of the appropriate test article approximately 2 hours after their lunch every day for 5 days. All daily doses were administered at the testing facility. Each subject was weighed prior to dosing so that the dose (by weight) of test materials was administered based upon the body weight of the individual (to the nearest kilogram) at the time of dosing. "High", "mid" and "low" dose levels selected were based on a weight of 70 kilogram subjects on a g/kg of body weight basis. Subjects returned to the clinic on the following Monday (7<sup>th</sup> day of study) to return their diaries and a final blood glucose determination was obtained.

Any subject experiencing severe diarrhea was discontinued following the day of occurrence. Others which flatulence and moderate diarrhea for two (2) consecutive days were also discontinued.

## **RESULTS**

Tables Ia and IIa through IIe summarize G.I. effects and blood glucose determinations on an individual dose/subject basis.

All of the Hystar test products produced adverse gastrointestinal effects at the high dose (2.1 g/kg).

Hystar 6075 (Sample G-37-2) did not produce these effects at the low dose and caused G.I. side effects in only one (1) subject on the mid-dose after the second dose on day 2.

Hystar 7000 (Sample G-37-1) caused severe symptoms in 12 subjects at all dose levels so that only one completed the five days of dosing. Note: thirteen (13) subjects were enrolled in this group.

Hystar HM-75 (Sample G-37-3) caused severe symptoms in 3 of the 12 subjects (4 subjects on high dose, 3 subjects on mid dose and 1 subject on low dose).

All subjects taking the sugar/corn syrup control were able to complete the study while one (1) of four (4) taking sorbitol was discontinued.

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Table I indicates those excused from the study because of severe gastrointestinal tract disturbances. There were twelve (12) subjects in group G-37-1 (Hystar 7000), five (5) in group G-37-2 (Hystar 6075), eight (8) in group G-37-3 (Hystar HM-75) and none in group G-37-5 (sugar/corn syrup). One (1) of four (4) subjects in group G-37-4 (sorbitol) was discontinued.

Blood sugars were relatively equivalent in all groups at all time intervals except that Hystar 6075 (sample G-37-2) seemed to produce higher levels at 30 and 60 minutes post-dosing at all three dose levels. One subject in this group had abnormally high levels (day 1) and skewed the average.

## **CONCLUSIONS**

Although the numbers of test subjects on each group and sub-group were not of sufficient size to permit appropriate statistical evaluation, it appears that sugar/corn syrup (group G-37-5) elicited the highest mean blood sugars at 30 and 60 minutes post dosing. Hystar 6075 (group G-37-2) seemed to cause higher levels at 30 and 60 minutes post dosing when compared with the two other Hystar compounds (see Table II).

It is concluded that Hystar 6075 is less likely to produce gastrointestinal disturbance than sorbitol, Hystar 7000 or Hystar HM-75. Both the sorbitol and sugar/corn syrup, controls, produced the least G.I. effects.

Within the limitations of group size, it appears that Hystar 7000, Hystar 6075 and Hystar HM-75 do not cause abnormal blood sugar curves and are similar (at 0.7 g/kg) to sorbitol. Sugar/corn syrup appeared to raise blood sugars to a higher degree than the other test articles.

**TABLE I**  
**EXCUSED FROM STUDY BECAUSE OF SEVERE G.I. TRACT**  
**DISTURBANCES**

**LAST DOSE**

Group ID	Day 1	Day 2	Day 3	Day 4	Day 5	Totals
<b>G-37-1 (Hystar 7000)</b>						
No. of Subs. A (High)						
B (Med)	4					4
C (Low)	3	1				4
	2	1	1			4
<b>G-37-2 (Hystar 6075)</b>						
No. of Subs. A						
B	2	2				4
C		1				1
						0
<b>G-37-3 (Hystar HM-75)</b>						
No. of Subs. A						
B	1	3				4
C			1	2		3
				1		1
<b>G-37-4 (Sorbitol)</b>						
No. of Subs. A						0
B						0
C	1					1
<b>G-37-5 (Sugar/Corn syrup)</b>						
No. of Subs. A						0
B						0
C						0

**TABLE IIa**  
**BLOOD GLUCOSE DETERMINATIONS (mg/dl)**  
**Hystar 7000 (G-37-1)**

Subj No.	Dose Level	DAY 1				DAY 2				DAY 3			
		B	30	60	180	B	30	60	180	B	30	60	180
7 13 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100 101 102 103 104 105 106 107 108 109 110 111 112 113 114 115 116 117 118 119 120 121 122 123 124 125 126 127 128 129 130 131 132 133 134 135 136 137 138 139 140 141 142 143 144 145 146 147 148 149 150 151 152 153 154 155 156 157 158 159 160 161 162 163 164 165 166 167 168 169 170 171 172 173 174 175 176 177 178 179 180 181 182 183 184 185 186 187 188 189 190 191 192 193 194 195 196 197 198 199 200 201 202 203 204 205 206 207 208 209 210 211 212 213 214 215 216 217 218 219 220 221 222 223 224 225 226 227 228 229 230 231 232 233 234 235 236 237 238 239 240 241 242 243 244 245 246 247 248 249 250 251 252 253 254 255 256 257 258 259 260 261 262 263 264 265 266 267 268 269 270 271 272 273 274 275 276 277 278 279 280 281 282 283 284 285 286 287 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538 539 540 541 542 543 544 545 546 547 548 549 550 551 552 553 554 555 556 557 558 559 560 561 562 563 564 565 566 567 568 569 570 571 572 573 574 575 576 577 578 579 580 581 582 583 584 585 586 587 588 589 590 591 592 593 594 595 596 597 598 599 600 601 602 603 604 605 606 607 608 609 610 611 612 613 614 615 616 617 618 619 620 621 622 623 624 625 626 627 628 629 630 631 632 633 634 635 636 637 638 639 640 641 642 643 644 645 646 647 648 649 650 651 652 653 654 655 656 657 658 659 660 661 662 663 664 665 666 667 668 669 670 671 672 673 674 675 676 677 678 679 680 681 682 683 684 685 686 687 688 689 690 691 692 693 694 695 696 697 698 699 700 701 702 703 704 705 706 707 708 709 710 711 712 713 714 715 716 717 718 719 720 721 722 723 724 725 726 727 728 729 730 731 732 733 734 735 736 737 738 739 740 741 742 743 744 745 746 747 748 749 750 751 752 753 754 755 756 757 758 759 760 761 762 763 764 765 766 767 768 769 770 771 772 773 774 775 776 777 778 779 780 781 782 783 784 785 786 787 788 789 790 791 792 793 794 795 796 797 798 799 800 801 802 803 804 805 806 807 808 809 810 811 812 813 814 815 816 817 818 819 820 821 822 823 824 825 826 827 828 829 830 831 832 833 834 835 836 837 838 839 840 841 842 843 844 845 846 847 848 849 850 851 852 853 854 855 856 857 858 859 860 861 862 863 864 865 866 867 868 869 870 871 872 873 874 875 876 877 878 879 880 881 882 883 884 885 886 887 888 889 890 891 892 893 894 895 896 897 898 899 900 901 902 903 904 905 906 907 908 909 910 911 912 913 914 915 916 917 918 919 920 921 922 923 924 925 926 927 928 929 930 931 932 933 934 935 936 937 938 939 940 941 942 943 944 945 946 947 948 949 950 951 952 953 954 955 956 957 958 959 960 961 962 963 964 965 966 967 968 969 970 971 972 973 974 975 976 977 978 979 980 981 982 983 984 985 986 987 988 989 990 991 992 993 994 995 996 997 998 999 1000 1001 1002 1003 1004 1005 1006 1007 1008 1009 1010 1011 1012 1013 1014 1015 1016 1017 1018 1019 1020 1021 1022 1023 1024 1025 1026 1027 1028 1029 1030 1031 1032 1033 1034 1035 1036 1037 1038 1039 1040 1041 1042 1043 1044 1045 1046 1047 1048 1049 1050 1051 1052 1053 1054 1055 1056 1057 1058 1059 1060 1061 1062 1063 1064 1065 1066 1067 1068 1069 1070 1071 1072 1073 1074 1075 1076 1077 1078 1079 1080 1081 1082 1083 1084 1085 1086 1087 1088 1089 1090 1091 1092 1093 1094 1095 1096 1097 1098 1099 1100 1101 1102 1103 1104 1105 1106 1107 1108 1109 1110 1111 1112 1113 1114 1115 1116 1117 1118 1119 1120 1121 1122 1123 1124 1125 1126 1127 1128 1129 1130 1131 1132 1133 1134 1135 1136 1137 1138 1139 1140 1141 1142 1143 1144 1145 1146 1147 1148 1149 1150 1151 1152 1153 1154 1155 1156 1157 1158 1159 1160 1161 1162 1163 1164 1165 1166 1167 1168 1169 1170 1171 1172 1173 1174 1175 1176 1177 1178 1179 1180 1181 1182 1183 1184 1185 1186 1187 1188 1189 1190 1191 1192 1193 1194 1195 1196 1197 1198 1199 1200 1201 1202 1203 1204 1205 1206 1207 1208 1209 1210 1211 1212 1213 1214 1215 1216 1217 1218 1219 1220 1221 1222 1223 1224 1225 1226 1227 1228 1229 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**TABLE II a**  
**BLOOD GLUCOSE DETERMINATIONS (mg/dl)**  
**HYSTAR 7000 (G-37-1)**

Sub	Dose Level	DAY 4				DAY 5				DAY 7
		B	30	60	180	B	30	60	180	
16	(High) 2.1 mg/kg									
17	A	-	-	-	-	-	-	-	-	-
18	A	-	-	-	-	-	-	-	-	-
19	A	-	-	-	-	-	-	-	-	-
	X=	-	-	-	-	-	-	-	-	-
	(Middle) 1.4mg/kg									
	B	-	-	-	-	-	-	-	-	-
	B	-	-	-	-	-	-	-	-	-
	B	-	-	-	-	-	-	-	-	-
	X=	-	-	-	-	-	-	-	-	-
	(Low) 0.7 mg/kg									
20	C	156	154	107	73	80	101	86	91	64
21	C	-	-	-	-	-	-	-	-	-
22	C	-	-	-	-	-	-	-	-	-
23	C	-	-	-	-	-	-	-	-	-
24	C	-	-	-	-	-	-	-	-	-
	X=	156	154	107	73	80	101	86	91	64

**B = Baseline**  
**30 = 30 minutes post-dosing**  
**60 = 60 minutes post-dosing**  
**180 = 180 minutes post-dosing**

**- = discontinued**  
**x= mean of all subjects**  
**glucose determinations**  
**(by group)**

000247

**TABLE II b**  
**BLOOD GLUCOSE DETERMINATIONS (mg/dl)**  
**HYSTAR 6075 (G-37-2)**

Subj No.	Dose Level	DAY 1				DAY 2				DAY 3			
		B	30	60	180	B	30	60	180	B	30	60	180
	<b>(High) 2.1 mg/kg</b>												
27	A	102	201	215	92	-	-	-	-	-	-	-	-
33	A	117	136	88	64	-	-	-	-	-	-	-	-
41	A	80	137	106	72	79	138	114	55	-	-	-	-
47	A	88	152	179	108	90	109	116	138	-	-	-	-
	X=	97	154	147	84	85	124	116	97	-	-	-	-
	<b>(Middle) 1.4mg/kg</b>												
23	B	69	144	142	102	83	86	117	97	107	122	100	69
49	B	116	154	146	160	79	116	99	90	128	131	154	93
	B	154	159	105	127	91	153	152	89	-	-	-	-
	B	81	123	117	114	69	172	203	82	65	116	125	56
	X=	105	145	128	126	73	132	143	90	100	123	126	73
	<b>(Low) 0.7 mg/kg</b>												
31	C	114	115	103	53	91	139	155	64	87	96	100	90
37	C	68	103	72	66	70	75	65	87	70	122	79	61
45	C	74	111	142	64	55	114	82	51	79	100	137	58
51	C	77	139	98	93	85	143	144	66	90	154	119	78
	X=	83	117	104	70	75	118	112	67	82	118	109	72

**B = Baseline**

**30 = 30 minutes post-dosing**

**60 = 60 minutes post-dosing**

**180 = 180 minutes post-dosing**

**- = discontinued**

**x = mean of all subjects glucose  
determinations (by group)**

**000248**

**TABLE II b**  
**BLOOD GLUCOSE DETERMINATIONS (mg/dl)**  
**HYSTAR 6075 (G-37-2)**

Subj ID	Dose Level	DAY 4				DAY 5				DAY 7
		B	30	60	180	B	30	60	180	
	(High) 2.1 mg/kg									
27	A	-	-	-	-	-	-	-	-	
38	A	-	-	-	-	-	-	-	-	
41	A	-	-	-	-	-	-	-	-	
47	A	-	-	-	-	-	-	-	-	
	X=	-	-	-	-	-	-	-	-	
	(Mid) 1.4 mg/kg									
	B	87	107	97	85	73	140	84	74	62
	B	67	131	145	93	109	117	147	95	121
	B	-	-	-	-	-	-	-	-	-
	B	71	149	197	71	107	106	149	88	96
	X=	75	129	146	83	96	121	127	86	93
	(Low) 0.7 mg/kg									
34	C	89	152	113	75	78	87	146	55	82
37	C	54	130	100	75	72	85	119	103	82
43	C	70	111	123	58	71	125	52	62	66
50	C	110	140	132	93	85	154	147	97	86
	X=	81	134	117	75	77	113	116	79	79

**B = Baseline**

**30 = 30 minutes post-dosing**

**60 = 60 minutes post-dosing**

**180 = 180 post-dosing**

**- = discontinued**

**x = mean of all subjects glucose  
determinations (by group)**

**000249**

**TABLE II c**  
**BLOOD GLUCOSE DETERMINATIONS (mg/dl)**  
**HYSTAR HM-75 (G-37-3)**

Subj No	Dose Level	DAY 1				DAY 2				DAY 3			
		B	30	60	180	B	30	60	180	B	30	60	180
	(High) 2.1mg/kg												
28	A	120	125	88	81	-	-	-	-	-	-	-	-
34	A	91	145	88	93	78	105	99	64	-	-	-	-
42	A	92	104	91	93	96	120	102	84	-	-	-	-
46	A	81	130	102	73	85	126	117	76	-	-	-	-
	X=	96	126	92	86	86	120	106	75	-	-	-	-
	(Mid) 1.4mg/kg												
36	B	91	79	123	112	86	105	86	59	68	123	75	64
38	B	82	115	128	117	101	109	125	117	85	91	90	97
44	B	80	125	117	98	98	129	140	79	80	125	117	98
50	B	122	104	112	91	90	124	136	73	71	124	121	114
	X=	94	106	120	105	93	117	122	82	79	116	101	94
	(Low) 0.7mg/kg												
52	C	157	171	171	118	146	192	186	113	123	150	156	108
58	C	94	122	118	85	83	113	112	71	66	102	107	72
48-R	C	116	98	84	67	81	87	83	90	99	92	125	67
52	C	73	113	106	98	81	100	83	107	117	103	97	87
	X=	110	126	120	92	103	123	116	95	99	112	121	84

B = Baseline  
 30 = 30 minutes post-dosing  
 60 = 60 minutes post-dosing  
 180 = 180 minutes post-dosing

- = discontinued  
 x = means of all subjects  
 glucose determination (by group)

**TABLE II c**  
**BLOOD GLUCOSE DETERMINATION (mg/dl)**  
**HYSTAR HM-75 (G-37-3)**

Subj	Dose Level	DAY 4				DAY 5				DAY 7
		B	30	60	180	B	30	60	180	
	(High) 2.1mg/kg									
23	A	-	-	-	-	-	-	-	-	-
34	A	-	-	-	-	-	-	-	-	-
12	A	-	-	-	-	-	-	-	-	-
43	A	-	-	-	-	-	-	-	-	-
	X=	-	-	-	-	-	-	-	-	-
	(Mid) 1.4 mg/kg									
	B	67	110	96	88	-	-	-	-	-
	B	-	-	-	-	-	-	-	-	-
14	B	96	129	140	79	84	142	108	96	102
59	B	63	98	110	64	-	-	-	-	-
	X=	75	112	115	77	84	142	108	96	102
	(Low) 0.7 mg/kg									
32	C	126	139	166	84	189	183	201	92	76
33	C	-	-	-	-	-	-	-	-	-
16-R	C	86	112	111	67	104	97	109	68	78
32	C	69	109	84	81	62	79	65	92	114
	X=	90	120	120	77	118	119	125	84	89

**B = Baseline**  
**30 = 30 minutes post-dosing**  
**60 = 60 minutes post-dosing**  
**180 = 180 minutes post-dosing**

**- = discontinued**  
**x = means of all subjects**  
**glucose determinations**  
**(by group)**

**TABLE II d**  
**BLOOD GLUCOSE DETERMINATIONS (mg/dl)**  
**SORBITOL CONTROL (G-37-4)**

Subj. No.	Dose Level	DAY 1				DAY 2				DAY 3			
		B	30	60	180	B	30	60	180	B	30	60	180
	(High) 2.1 mg/kg					( NOT DOSED )							
	A												
	A												
	A												
	X=					( NOT DOSED )							
	(Mid) 1.4 mg/kg												
	B												
	B												
	B												
	X=												
	(Low) 0.7 mg/kg												
26	C	95	85	96	78	69	67	78	73	97	92	66	81
26	C	78	109	86	89	92	71	76	169	71	100	86	87
39	C	67	87	82	79	84	76	77	81	-	-	-	-
40	C	44	114	108	98	89	95	91	59	114	84	125	170
	X=	71	99	93	86	84	77	81	96	94	92	92	113

**B = Baseline**  
**30 = 30 minutes post-dosing**  
**60 = 60 minutes post-dosing**  
**180 = 180 minutes post-dosing**

**- = discontinued**  
**x = means of all subjects**  
**glucose determinations (by group)**

000252

**TABLE II d**  
**BLOOD GLUCOSE DETERMINATIONS (mg/dl)**  
**SORBITOL CONTROL (G-37-4)**

Dose Level	DAY 4				DAY 5				DAY 7
	B	30	60	180	B	30	60	180	FINAL READING
(High) 2.1mg/kg	(NOT DOSED)								
A									
A									
A									
X=	(NOT DOSED)								
(Mid) 1.4mg/kg									
B									
B									
B	(NOT DOSED)								
B									
X=									
(Low) 0.7mg/kg									
C	74	94	82	58	68	79	78	71	80
C	99	93	80	68	65	62	59	75	74
C	-	-	-	-	-	-	-	-	-
C	120	82	123	96	117	99	104	86	101
X=	97	90	98	77	83	80	80	77	85

B = Baseline  
 30 = 30 minutes post-dosing  
 60 = 60 minutes post-dosing  
 180 = 180 minutes post-dosing

- = discontinued  
 x= means of all subjects  
 glucose determinations (by group)

000253



**TABLE II e**  
**BLOOD GLUCOSE DETERMINATIONS (mg/dl)**  
**SUGAR/CORN SYRUP (G-37-5)**

Subj No	Dose Level	B	30	60	180	B	30	60	180	FINAL READ
2 8 14-R 20-R	(High) 2.1mg/kg									
	A	87	120	136	107	93	129	155	124	79
	A	78	232	197	98	67	185	209	66	129
	A	102	136	143	93	74	159	110	62	141
	A	109	185	182	82	76	146	155	97	-
	X=	94	168	155	95	77	155	157	87	116
4 10 16-R 22	(Mid) 1.4mg/kg									
	B	28	105	115	92	74	105	116	103	80
	B	83	145	132	72	97	141	133	76	81
	B	103	126	126	96	114	123	144	60	98
	B	95	129	125	82	70	187	137	70	84
	X=	77	126	125	86	89	139	133	77	88
11 12 13 24-R	(Low) 0.7mg/kg									
	C	60	123	107	69	71	163	109	59	60
	C	82	128	159	84	98	124	112	78	69
	C	120	213	144	99	101	169	203	120	73
	C	71	183	105	118	104	158	143	88	92
	X=	83	162	131	93	94	154	142	89	71

B = Baseline

- =

discontinued

30 = 30 minutes post-dosing  
subjects

x= means of all

60 = 60 minutes post-dosing  
determinations (by group)

glucose

180 = 180 minutes post-dosing

000254





DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration  
Washington, DC 20204

FD



October 23, 2000

Ms. Rani Thomas  
Director of Quality  
and Regulatory Affairs  
Grain Processing Corporation  
1600 Oregon Street  
Muscatine, IA 52761

Re: GRAS Notice (GRN) No. 000059

Dear Ms. Thomas:

The Food and Drug Administration (FDA) has received the notice, dated September 11, 2000, that you submitted on behalf of Grain Processing Corporation in accordance with the agency's proposed regulation, proposed 21 CFR 170.36 (62 FR 18938; April 17, 1997; Substances Generally Recognized as Safe (GRAS)). FDA received this notice on October 4, 2000 and designated it as GRN No. 000059.

The subject of the notice is hydrogenated starch hydrolysate. The notice informs FDA of the view of Grain Processing Corporation that hydrogenated starch hydrolysate is GRAS, through scientific procedures, for use as a flavoring agent and adjuvant, formulation aid, humectant, processing aid, stabilizer, thickener and texturizer.


In accordance with proposed 21 CFR 170.36(f), a copy of the information in your notice that conforms to the information described in proposed 21 CFR 170.36(c)(1) is available for public review and copying on the Office of Premarket Approval's homepage on the Internet (at <http://vm.cfsan.fda.gov/~lrd/foodadd.html>). If you have any questions about the notice, contact Dr. Rosalie Angeles at (202) 418-3107.

Sincerely yours,

Suzette Williams  
Division of Product Policy, HFS-205  
Office of Premarket Approval  
Center for Food Safety  
and Applied Nutrition

000368

(b) (5)



000369



**DEPARTMENT OF HEALTH AND HUMAN SERVICES**  
**Food and Drug Administration**  
**Memorandum of Telephone Conversation**

**Dates:** June, 2001 through September, 2001

**Between:** Linda Kahl, Ph.D. (HFS-206)

and

Rani Thomas, Grain Processing Corporation (GPC), (563)264-4681

**Subject:** GRAS Notice No. GRN 000059 (Hydrogenated Starch Hydrolysate (HSH))

During the period June 2001 through September, 2001, Rani Thomas called me several times about GRN 000059, for the use of HSH as a flavoring agent and adjuvant, formulation aid, humectant, processing aid, stabilizer and thickener, surface-finishing agent and texturizer in hard candy, soft candy, chewing gum, bakery products and ice cream. In her calls, she inquired about the status of FDA's evaluation of GRN 000059, whether it was permissible to market the ingredient before FDA completed its evaluation, and whether it was possible to withdraw the notice. This memorandum summarizes those conversations. In the end, Ms. Thomas decided to ask FDA to cease to evaluate GRN 000059.

On June 4, Ms. Thomas left a message and asked for an update on the status of FDA's evaluation of GRN 000059. I returned her call on June 5, and explained that OFAS needed to discuss GRN 000059 within the Center and the agency because of the issues raised by the carcinogenicity studies conducted with sugar alcohols that are components of HSH. On June 6, Ms. Thomas called and asked whether her firm could market its HSH product while the GRAS notice was pending, and noted that other manufacturers were selling similar HSH products. I explained that a GRAS notice is not a premarket notice and that GPC could have marketed its HSH product even before it submitted its GRAS notice.

On June 12, Ms. Thomas told me that she was not aware of any studies linking its HSH product to cancer. I asked Jason Dietz of OFAS to identify some literature that discussed studies conducted with one or more of the components of HSH, and he sent Ms. Thomas two publications (see letter dated June 25, 2001, from Jason Dietz to Rani Thomas).

On August 28, Ms. Thomas called again to ask about the status of FDA's evaluation of GRN 000059. I told her that it was still under consideration. She then asked whether I would let her know before we issued our letter if the letter would find no basis for a GRAS determination. She was concerned about the selective impact of such a letter on GPC, but not on competitors who were selling similar products. Given the circumstances, I made a commitment that I would inform her if the final conclusion of the agency was that the notice does not provide a basis for a GRAS determination. The next day, Ms. Thomas called and asked whether GPC would have an option to withdraw the notice rather than receive a letter that the notice did not provide a basis for a GRAS determination. I replied that this was an option. On September 4, Ms. Thomas asked whether GPC could still sell its HSH product if it withdrew its GRAS notice. Consistent with my response to her earlier question about marketing HSH, I said yes.

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Page 2 - Telephone conversation with


On September 10, Ms. Thomas called and asked whether I was aware that a carcinogenicity study on maltitol showed no carcinogenicity. I replied that FDA needed to consider the complete picture, which included studies other than the one she described.

During the months that these conversations were occurring, OFAS held internal discussions about GRN 000059 and concluded that OFAS should consult with the Office of Chief Counsel prior to responding to GRN 000059. This consultation had not happened by the time that OFAS received GPC's letter dated September 14, 2001, asking that FDA cease to evaluate GRN 000059.

~~Linda S. Kahl~~

Linda S. Kahl  
November 7, 2001

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DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration  
Washington, DC 20204

June 25, 2001

FD



Rani Thomas  
Director of Quality and Regulatory  
Grain Processing Corporation  
1600 Oregon St.  
Muscatine, IA 52761

Dear Ms. Thomas:

This responds to your telephone inquiry of June 14, 2001, requesting references concerning the carcinogenicity of sorbitol. Please find enclosed the two references that we discussed during our telephone conversation of June 21, 2001. The references discuss the potential for sorbitol and other sugar alcohols to cause proliferative lesions in the adrenal glands of rats.

The references enclosed are:

Lynch, B. S., Tischler, A. S., Capen, C., Munro, I. C., McGirr, L. M., McClain, R. M. (1996) Low digestible carbohydrates (polyols and lactose): significance of adrenal medullary proliferative lesions in the rat. *Regulatory Toxicology and Pharmacology*. 23:256-297.

Dills Jr., W. L. (1989) Sugar alcohols as bulk sweeteners. *Annual Review of Nutrition*. 9:161-186.

If you have any further questions concerning this matter, please do not hesitate to contact us.

Sincerely,

Jason Dietz  
Office of Food Additive Safety HFS-206  
Center for Food Safety  
and Applied Nutrition

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## SUGAR ALCOHOLS AS BULK SWEETENERS

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### INTRODUCTION

#### *Scope of this Review*

This review considers polyols as bulk sweeteners, i.e. substances used to replace sucrose in food manufacture and in the household setting to provide sweetness and body or bulk to foods and beverages. Although numerous polyols have been examined, only the six in which commercial interest is the greatest are considered here in detail: the monosaccharide polyols sorbitol,

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mannitol, and xylitol; the disaccharide polyols maltitol and lactitol; and an equimolar mixture of two disaccharide polyols, D-glucosyl- $\alpha$ -(1-1)-D-mannitol and D-glucosyl- $\alpha$ -(1-6)-D-glucitol, that is denoted Palatinit<sup>®</sup>. Maltitol is the major component of two additional products discussed along with maltitol: Malbit<sup>®</sup> (79.9-88.4% maltitol) and Lycasin<sup>®</sup> (53.8% maltitol); these products also contain sorbitol, maltotriitol, and other minor components. The interest in alternatives to sucrose has resulted in extensive research on the polyols in recent years (9, 12-14, 53, 87, 101, 104, 107, 108, 148, 169, 180). For aspects covered in other appropriate reviews, the reader is referred to those reviews and given capsule summaries here. In general, clinical uses of the polyols are not discussed.

The reasons for the great interest in the polyol sweeteners follow:

1. Their intrinsic sweetness is of the same order of magnitude as that of sucrose. This point is discussed below.

2. They are easily manufactured, as discussed in earlier reviews.

3. Sugar consumption has a number of real and perceived side effects (see review 54). In this regard, three potential advantages of the polyols have been noted (e.g. 180):

a. For a large portion of the general population, the use of polyols may potentially reduce the incidence of dental caries. This topic has been reviewed in detail for xylitol (13, 104, 107, 108) and to a lesser extent for the other polyols (107, 180).

b. Some polyols have reduced caloric content, a quality of potential interest in the management of obesity. The basis of this reduced caloric content differs for various polyols and is discussed below. Also considered below is that this reduced caloric content may be more apparent than real when the relative sweetness is also considered.

c. The polyols in general yield glucose at a slower rate than dietary glucose or sucrose, which results in flattened blood glucose curves. This point is of interest and perhaps value to diabetics. The particular applications involving the use of polyols as sucrose substitutes for diabetics are not discussed in this review; interested readers are referred to reviews on this specific topic and to earlier reviews on the polyols.

### *Current Regulatory Status*

Sorbitol is a direct food substance generally regarded as safe (GRAS). If consumption of a sorbitol-containing food is likely to result in a daily ingestion of more than 50 g, the label must contain the statement "Excess consumption may have a laxative effect." Mannitol is a food additive permitted on an interim basis pending further study. The same label noted above for sorbitol must be applied to any mannitol-containing food that could conceivably result in the ingestion of 20 g or more of mannitol in a single day. Both mannitol and sorbitol are widely used in food manufacture in the US.

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Xylitol is a food additive permitted for special dietary or nutritional uses provided that the amount used is not in excess of that needed to produce the intended effect. A proposed revocation was never finalized and is still pending. A select panel of experts was recently convened by the Life Sciences Research Office (LSRO) of the Federation of American Societies for Experimental Biology under contract to the FDA to consider the scientific evidence regarding xylitol (see review 101). The present use of xylitol in the United States is limited.

Lactitol, maltitol, and the maltitol-containing product Lycasin are the subjects of GRAS petitions received and filed by the FDA. The same panel of experts noted above for xylitol also considered lactitol in their deliberations (101). A petition concerning Palatinit has also been received by the FDA but had not been filed as of winter, 1989.

National food laws and regulations differ from country to country and so, too, does the status and use of individual polyols (see 12 regarding xylitol for example). No attempt is made here to summarize the status of polyols as food additives in countries other than the US. It appears that each of the polyols discussed herein is either utilized or has at least been petitioned for specific commercial applications in some European nations.

#### *Comparative Sweetness of the Polyols*

The majority of simple sugars and polyols elicit a taste response characterized as "sweet." Because of the interest in sucrose substitutes, a wide variety of compounds have been subjected to tests of comparative sweetness. The values reported for the sweetness of all compounds, including sucrose and the polyols, are based on subjective assay methods, and results vary with assay conditions. Slopes of the sweetness-concentration lines differ for different sweeteners, and the relationships are not always linear as can be seen in the report by Hoppe & Gassmann (78) for a wide variety of sweeteners, including several polyols. Furthermore, sweetness values for different compounds are differentially influenced by temperature, as seen, for example, in the results of Hyvönen et al (82) in a detailed study of the sweetness of fructose, glucose, and xylitol. Nevertheless the results reported by different groups in comparative sweetness studies on the polyols are quite consistent (38, 78, 113, 180). On the basis of sweetness per gram, the sweetest polyol of those considered here is xylitol, which is generally reported as being "isosweet" with sucrose. Sorbitol, mannitol, and maltitol and the maltitol products were somewhat less sweet, with values ranging from 45 to 90% of the sweetness of sucrose. Lactitol and Palatinit were the least sweet, with sweetness values reported from 25 to 50%.

Of greater potential interest with regard to the use of polyols as bulk sweeteners are the sweetness values as expressed per kilocalorie of available metabolic energy. Values for kcal/g vary depending on the dosage and

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experimental animal used, and few comparative studies are available. Results are probably best reviewed by Ziesenitz & Siebert (180). In humans, sorbitol and xylitol are reported as having caloric contents similar to that of sucrose, although high doses will most certainly result in decreased caloric values because these polyols are incompletely absorbed at higher dietary levels. Other polyols have reduced caloric values ranging from 40 to 70% of those reported for sucrose. For mannitol the reduced value probably reflects the incomplete metabolism of mannitol by L-iditol dehydrogenase and the incomplete reabsorption of mannitol by the kidney (for review see 169). For the disaccharide polyols, the low caloric values reflect slow rates of hydrolysis in the digestive tract and the involvement of intestinal microflora, as discussed below and in the review by Ziesenitz & Siebert (180). In any case, the polyols with reduced caloric contents tend to be those with reduced sweetness compared with sucrose, so that when sweetness is expressed per kcal, the six polyols are actually quite similar.

## ABSORPTION AND METABOLISM OF THE POLYOLS

### *Monosaccharide Polyols*

The absorption and metabolism of the monosaccharide polyols are thoroughly reviewed elsewhere and only summarized here. Sorbitol, mannitol, and xylitol are all absorbed from the digestive tract by passive diffusion and therefore enter the circulation less rapidly than do glucose or fructose. A portion of the ingested polyol, particularly at high doses, reaches the lower digestive tract, with the resulting involvement of the intestinal flora. In laboratory animals and humans, large doses result in diarrhea, although adaptation involving changes in intestinal flora frequently occurs over time. Some investigations have reported that xylitol is better tolerated than are the hexitols. In mice and rats, high levels of dietary monosaccharide polyol are associated with the cecal enlargement characteristic of any slowly absorbed carbohydrate, and in humans there are the increases in breath hydrogen or methane characteristic of colonic microfloral involvement.

The first step in the metabolism of all three monosaccharide polyols involves oxidation by the hepatic L-iditol dehydrogenase to the corresponding 2-ketose, D-fructose for sorbitol and mannitol and to D-xylulose for xylitol. For sorbitol and xylitol this oxidation is extensive, and only small quantities of polyol are excreted in the urine. Mannitol is a poor substrate for the enzyme, and significant portions of ingested mannitol are excreted. Most of the fructose and xylulose thus formed are phosphorylated by fructokinase and xylulokinase, respectively. The subsequent metabolism of the resulting phosphate esters by the fructose and pentose shunt pathways, respectively, is well documented. For xylitol a minor pathway has been proposed (19, 84) that

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involves the phosphorylation of D-xylulose to xylulose-1-phosphate by fructokinase. This pathway does not play a major role in the overall metabolism of xylitol but may play a role in the relationship between xylitol and oxalate and is thus discussed below (see "Xylitol and Oxalate").

### *Disaccharide Polyols*

Disaccharide polyols are mostly digested to hexoses and monosaccharide polyols, which are then absorbed. Traces of the intact disaccharide polyols are absorbed as evidenced by the small but measurable quantities of each recovered in the urine of rats and humans following ingestion (Table 1). In this regard Hosoya (79) demonstrated the transport of labelled maltitol in everted sacs of rat intestine.

Hydrolysis of the disaccharide polyols by the saccharidases of the intestine and other tissues has been studied extensively. Early studies performed with human salivary  $\alpha$ -amylase and  $\alpha$ -glucosidase from the small intestine of humans (172) and rats (173, 179) demonstrated that maltitol was a poor substrate and a weak inhibitor for these enzymes. Similar results were reported for the intestinal  $\alpha$ -glucosidase and the two polyols of Palatinit (57). These results with maltitol have been confirmed and extended with highly purified preparations of the enzyme from rats (134). The relative maximal rates of hydrolysis for sucrose, maltose, maltitol, glucosyl-(1-1)mannitol, glucosyl-(1-6)glucitol, Palatinit, and Malbit were 280, 900, 112, 32, 67, 35, and 250-310 nmol/min/mg protein, respectively (57, 179). Tsuji et al (162) observed that the relative rates of hydrolysis of several of these compounds corresponded to their bioavailability as measured by the transmural potential differences produced by the  $\text{Na}^+$ -dependent-glucose transport in rat everted jejunal sac preparations. Most recently the rates of hydrolysis of disaccharides and disaccharide polyols were compared using human intestinal biopsies. Average rates of hydrolysis for maltose, sucrose, lactose, maltitol, Palatinit, and lactitol were 172, 42.9, 20.2, 19.1, 2.5, and 0.34  $\mu\text{mol/min/g}$  respectively at 37°C. Furthermore, maltitol and Palatinit but not lactitol inhibited glucose release approximately 25% from maltose when present at the same concentration as the disaccharide (116).

The saccharidase hydrolysis products can be either absorbed directly or metabolized subsequently by the intestinal flora. Absorption of the hexitol products was discussed above and is apparent from the free hexitols measured in the urine (Table 1) and the blood (147) following disaccharide polyol ingestion. The glucose formed from maltitol or Palatinit and the galactose formed from lactitol are rapidly absorbed into the blood stream, as evidenced in the small but detectable rises in blood glucose in rats consuming Palatinit (114) and in humans consuming maltitol (94, 147) or Palatinit (150, 157) and by the rise in blood galactose in humans ingesting lactitol (175).

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Table 1 Excretion of the disaccharide polyols in rats and humans

Polyol (Ref.)	Subject	Dose <sup>a</sup>	Polyol excreted in <sup>b</sup>			
			Urine		Feces	
			unchanged	hexitol	unchanged	hexitol
Palatinit (178)	rat	5% diet	0.11	0.09	6.0	8.3
	rat	10% diet	0.04	0.04	7.8	9.4
	rat, germ-free	5% diet	89	0.94	50	2.0
	rat, germ-free	10% diet	114	3.42	275	1.4
Palatinit (114)	rat	5% diet	n.r.	n.r.	18.7	21.3
Maltitol (94)	rat	1.0 g	~25	<5	~50	~15
	rat, germ-free	1.0 g	~210	~15	~90	~20
Maltitol (98)	rat	1.0 g	8	0.6	5	3
	rat	2.0 g	15	2	144	180
	rat	2.0 g	18.9	0.08	46.6	10.0
	rat, germ-free	2.0 g	211.6	10.4	86.5	13.1
Lactitol (95)	rat	2 mg	0.0	0.23	0.0	0.14
Palatinit (150)	human	50 g	n.r.	n.r.	n.r.	78
	human	100 g	n.r.	n.r.	n.r.	94
Maltitol (147)	human	3×10 g	350-400	422	33	21
Lactitol (175)	human	50 g	n.r.	n.r.	n.r.	33 mg/dL

<sup>a</sup> Single doses were administered by stomach tube.<sup>b</sup> In mg/24 h unless indicated. The notation n.r. indicates that data were not reported

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The importance of the intestinal flora in the metabolism of the disaccharide polyols is supported by several lines of evidence. First, the excretion patterns for disaccharide polyols and their hexitol products in normal and germ-free rats (Table 1) strongly support intestinal flora involvement. For maltitol and Palatinit, significantly more mono- and disaccharide polyol occurs in urine and feces of the germ-free animals than in comparable normal animals when fed the disaccharide polyols. Second, in rodents lactitol (135, 167), maltitol (79), and Palatinit (114, 178) cause cecal enlargement attributable to slowly absorbed carbohydrate. Third, the formation of hydrogen gas in humans has been studied with Palatinit (47) and with lactitol (56, 166). This gas formation is usually considered to indicate colonic involvement in metabolism. Fourth, a significant conversion of maltitol into fatty acids was observed in rat and human feces, which indicates that this polyol was fermented in the gastrointestinal tract (125). Last, the caloric values for lactitol (23, 45, 166), maltitol (79, 92, 125), and Palatinit (57, 94, 114, 157) were less than those for sucrose in animals and humans (see also 180 and references therein), probably a reflection of the microfloral utilization. In this regard Ziesenitz (177) has performed some interesting experiments with the carbohydrate-deficient diet assay system of Karimzadegan et al (89), which allows one to evaluate how a particular dietary component is available as carbohydrate. Mannitol, sorbitol, D-glucosyl- $\alpha$ (1-1)-D-mannitol, and D-glucosyl- $\alpha$ (1-6)-D-glucitol were metabolized to the extent of 6, 20, 39, and 42% as carbohydrate, respectively. The differences between these values and the percentage utilization values will ultimately be useful in evaluating the availability of fatty acids formed during cecal fermentation.

The major saccharide absorption products from the disaccharide polyols appear to be hexitol, hexose, and unchanged disaccharide polyol (Table 1). The metabolism of the hexitol portions was discussed briefly above, and the metabolic pathways for galactose (from lactitol) and glucose (from maltitol and Palatinit) are well known and need not be discussed here. The disaccharides are nearly inert as judged by their excretion in the urine following injection (92, 150) and by the fact that they are non- or poor substrates for L-iditol dehydrogenase (114) or aldose reductase (41).

### POLYOL SWEETENERS AND DENTAL CARIES

Recent reviews describe the extensive work done on the relationship of polyols, especially xylitol, and dental caries (13, 104, 107, 108, 180). The most recent material is summarized below.

Dental caries is mediated by bacteria that accumulate in large masses and contribute to dental plaque. Accumulation of these bacteria is facilitated by the extracellular biosynthesis of insoluble glucans that increase cellular

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adhesive properties and serve as a matrix for plaque formation. Fermentation of common dietary carbohydrate by plaque bacteria acidifies the plaque milieu, dissolves calcium and phosphate from the tooth enamel, and eventually forms a cavity. Dietary sucrose is believed to play a major role in caries formation (see reviews 54, 142). Polyol sweeteners can help prevent dental caries by inhibiting the fermentation of dietary carbohydrate and the formation of insoluble glucan. In general, all of the polyol sweeteners are significantly less cariogenic than sucrose, and xylitol may actually be cariostatic or anticariogenic.

### *Sorbitol and Mannitol*

There is particular interest in sorbitol, as it has long been used as a humectant and binding agent to enhance the texture and shelf-life of dentifrice products (110). Sorbitol and mannitol are slowly fermented by oral microorganisms, and this decreases the acidification of the plaque. Long-term use of sorbitol may lead to metabolic adaptation by the oral microflora, and even traces of glucose have been reported to repress sorbitol metabolism. Formation of the soluble and insoluble polysaccharides of *Streptococcus mutans* was considerably less with sorbitol than with sucrose or fructose, and decreased adhesiveness of the microbial cells to glass surfaces has been observed.

In studies of rats under various conditions, dietary sorbitol and mannitol showed significantly less cariogenic activity than sucrose. In humans, the low cariogenicity of sorbitol appears to be supported by the beneficial results of good oral hygiene, which frequently involves a dentifrice containing sorbitol (e.g. 110).

### *Xylitol*

Xylitol is fermented only very slowly by oral bacteria and is generally not catabolized into acid products. Oral microflora do not appear able to acquire the ability to utilize xylitol.

Animal studies have shown that rats fed xylitol had a low incidence of caries, significantly below that of rats fed other polyols, which was in turn below that of rats fed sucrose. The question as to whether xylitol was merely noncariogenic or was actively anticariogenic was studied in several laboratories. In general the results showed that under certain conditions xylitol significantly reduces the cariogenic potential of sucrose. In humans much work has been done with xylitol and is reported in the reviews cited earlier. In general the conclusions reached in the extensive Turku sugar studies (145) concerning xylitol's cariostatic or anticariogenic properties were confirmed in more recent studies in Thailand (16), Hungary (143, 144), and French Polynesia (16, 88).

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**MECHANISMS OF THE CARIOSTATIC EFFECTS OF XYLITOL** The cariostatic or anticariogenic effects of xylitol are quite complex. It appears that more than one mechanism exists. It is not known at this time which of the effects noted below are clinically relevant. Much of this material can be found in the earlier review by Mäkinen & Scheinen (108) and other more recent reviews (13, 107).

On the microbial level, xylitol may produce its anticariogenic effects by several processes. In addition to being slowly fermented by oral bacteria, xylitol inhibits the acid formation from glucose in *S. mutans* much more effectively than any other polyol (180). In *S. mutans* and other oral bacteria the first metabolic step in the utilization of many polyols involves a phosphoenolpyruvate-dependent phosphorylation step linked to polyol or sugar uptake rather than a dehydrogenation reaction (124, 127). These phosphorylation-uptake systems involve several proteins, and the transport protein components are responsible for the substrate specificity of the process (124, 127). Quite probably, polyols exert some of their inhibitory effects on acid production in this phosphorylation-uptake process. In addition certain oral bacteria apparently may convert xylitol to a toxic metabolite, namely xylitol-5-phosphate (4, 73, 159, 161). Adaptation to xylitol leads to xylitol-resistant strains of bacteria, however (50, 160), even though cariostatic effects remain (20, 110).

Xylitol also inhibits the formation of the insoluble polysaccharides of *S. mutans*, although the synthesis of the soluble polysaccharides is increased by xylitol (154). The changes in polysaccharide synthesis in the presence of xylitol resulted in a decrease in the adhesive properties of the bacteria. The synthesis of the insoluble polysaccharides by *S. mutans* appears to involve multiple extracellular glucosyl transferases (59). Some of these enzymes are inhibited by polyols (G. Siebert and F. Frosthuber, unpublished observations cited in 180), although xylitol exerted only slight inhibitory effects in their system (G. Siebert, personal communication).

Most recently, Beckers (20) studied the growth and metabolism of xylitol-sensitive and xylitol-resistant mutants of *S. mutans*. While differences in colony establishment time in vivo and growth rate in vitro could be observed, the formation of fissure caries lesions was inhibited by xylitol regardless of whether the xylitol-resistant or xylitol-sensitive strain was used. This result suggests that some of the anticariogenic properties of xylitol may be independent of oral microflora. Specific changes in salivary parameters occur in response to xylitol and may play a role in the natural defenses against dental caries. Many were observed during the original Turku sugar studies (145) and are noted extensively in review articles (13, 104, 107, 108). Among the changes observed in saliva are increases (a) in volume produced by sweeteners and more specifically in glycine and the basic amino acids, (b) in total

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salivary protein, (c) in the activities of saccharidases and other enzymes, and (d) in the level of thiocyanate ion—all of which may play a role in the natural defense against caries. More recently an increase in ammonia has also been noted to occur concomitantly with the decrease in lactic acid formation (105, 155).

Last, the physical chemical properties of xylitol may be involved. Polyols in general protect proteins from denaturation (see review in 105), a factor that may play a role in changes in the salivary environment noted above. Xylitol and other polyols also form strong calcium complexes (105, 109) that prevent the precipitation of calcium phosphate. Xylitol did not enhance the rehardening of surface-softened enamel under conditions favoring rehardening (152). On the other hand, high xylitol concentrations prevented the demineralization of enamel in vitro (2). In vivo the same effect was observed on surface enamel but not in fissures (153). This latter effect was attributed to the absence of plaque on the enamel surface.

### *Disaccharide Polyols*

Only recently have the disaccharide polyols been considered sweeteners; as a result they have not been subject to the same scrutiny as have the monosaccharide polyols. The disaccharide polyols all appear to be less cariogenic than sucrose and possibly also less than the hexitols (see reviews 107, 180). In bacterial studies they are fermented slowly by oral microflora (22, 43, 52, 163). They reduced the incidence of caries in laboratory animals (51, 52, 55, 74, 90, 91, 164, 165) and, in the few studies performed thus far, in humans (24). With regard to the mechanisms of their effects, the disaccharide polyols, besides being slowly fermented by oral bacteria, appear to inhibit acid production from glucose (180 and references therein).

## PHYSIOLOGIC AND TOXICOLOGIC EFFECTS OF THE POLYOLS

Numerous studies have been performed with the polyols in animals and humans. The sections below discuss new information obtained in certain areas and recent work on the reported occurrence of tumors in laboratory animals consuming polyol-containing diets. For the purpose of discussion, the effects are divided into those reported for many if not all of the polyols in question and those unique to individual polyols. Note that in general polyols have low toxicities and mutagenicities as discussed in earlier reviews.

### *Effects Common to Many of the Polyols*

**EFFECTS OF POLYOLS ON MINERAL METABOLISM** When 20% dietary xylitol, mannitol, or galactitol are included in rat diets, urinary calcium

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excretion increases (46, 65, 140). Similarly, the presence of 10% sorbitol in mouse diets results in an increase in the levels of blood calcium and in calcium excretion (103). This increased calcium excretion stemmed from increased dietary calcium absorption and not loss of skeletal calcium (67). Similar effects were observed with lactitol (1, 9, 101). Earlier investigations had reported that other slowly absorbed carbohydrates such as lactose (21, 42, 77, 96) and the modified starches (37, 77, 93; see review 100), but not the nonabsorbable carbohydrates found in fiber (see review 99), also increased calcium absorption in rats. The effects of lactose on calcium absorption have also been noted in humans (34, 176), although the effects are considerably smaller than in rats.

The absorption of other minerals is enhanced by polyols in the diet. In rats fed diets with high polyol levels, an increase in iron absorption has been observed (62, 63, 65, 75). With sorbitol, a similar effect was reported in humans (58, 102). In the rat the increase in iron absorption was accompanied by an increase in duodenal xanthine oxidase and ferroxidase (64). Also, dietary xylitol increases the absorption of dietary lead in female mice but not in male mice or cockerels (115). Last, in mice but not rats, dietary xylitol increased the intestinal absorption and urinary excretion of oral oxalate (136, 139).

For purposes of discussion the effects are treated here as if similar mechanisms operate for each of the metal ions and as if the effects produced by lactose, starches, and the polyols are the same. Most of the mechanistic work has been performed with calcium, for which there are two independent uptake processes: a physiologically regulated, vitamin-D-regulated transcellular process and a nonsaturable, vitamin-D-independent process (30, 171). A variety of evidence supports the statement that slowly absorbed carbohydrates affect the nonsaturable process. First, the lactose effect was observed to occur in the ileum (96), where calcium is absorbed only by the nonsaturable process (122). Second, the xylitol (67) and lactose (112) effects are vitamin D independent. Third, the levels of carbohydrate needed to produce the effect are high enough to cause the cecal enlargement in rodents that occurs when unabsorbed carbohydrate is present in the lower intestine (see references above and 121).

Outside of this conclusion, the mechanisms by which the polyol-lactose effects are produced are not clear. Several possible mechanisms for the effects of polyols and lactose on mineral absorption have been reviewed (101, 128-130); they include complex formation involving the mineral in question and the polyol or sugar (32, 61, 109), the removal of an energy barrier to calcium movement (70), changes in the transport potential of the intestine (111), changes in luminal calcium concentrations due to water absorption (117, 174), the mechanical extension of the intestine by the hyperosmolar

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polyol solution in the intestinal lumen (101), and the acidification of the intestinal contents (1). Any and all of these mechanisms may eventually be found to play a role in the polyol-induced increases in mineral uptake. Any discussion of mechanism must ultimately consider the following information, some of which was discussed above.

First, the effect requires absorbable carbohydrate or polyol in the intestine. Glucose, which normally does not reach the lower gastrointestinal tract, does enhance calcium absorption in ligated loops (123) and in vivo if introduced by perfusion (174). Non- or poorly metabolized but absorbable substances like xylose (123) and mannitol (65, 117) were also effective. Note that these latter substances are metabolized by intestinal flora and absorbed from the intestine.

Second, the lactose effect's dependence on lactase (34) suggests that the carbohydrate needs to be in monosaccharide form or in the form in which it will be absorbed.

Third, those minerals that formed complexes of intermediate strength with the polyols were those with enhanced absorption, while those with stronger or weaker interactions were unaffected (28, 61). There is no evidence for cotransport, however, and the lactose effect in rat gut sacs required only preincubation with lactose and was not produced by the simultaneous presence of lactose (3).

**POLYOLS AND THE RAT ADRENAL GLAND** One of the observations of the so-called Huntingdon studies was the incidence of adrenal medullary hyperplasias associated with xylitol feeding in rats (48, 49, 80). More recently, long-term feeding studies have noted an increased incidence of adrenal medullary hyperplasia and pheochromocytoma in rats fed polyol-containing diets (5, 9, 128-130, 151). These lesions have not been seen in any other species.

The mechanisms linking the hyperplastic changes observed in rats to the polyols in their diets are not known but appear to be linked to the changes in calcium metabolism discussed above. In general, high levels of dietary polyol or lactose are associated with increased catecholamine levels (14, 25, 26, 66, 106), but controversy exists as to the effect on actual catecholamine synthesis and as to whether the effects observed are primary or secondary to other homeostatic changes. Bär has reported that decreasing the concentration of calcium in the diets of rats fed 20% xylitol reverses the increases in calcium excretion noted above and simultaneously decreases the catecholamine levels in adrenal glands of rats and the incidence of proliferative changes (5, 14; also see review 101). Whether the polyol- or lactose-produced effects on the adrenal medulla are mediated by calcium itself or are mediated by accompanying changes in the calcium homeostatic hormones is not known. Indirect evidence linking calcium to adrenal function in rats has been reported

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(see review 14). Vitamin D deficiency reportedly lowers catecholamine levels (8, 29), and hypercalcemia has been observed to increase blood catecholamine levels (156).

Also, high levels of dietary xylitol resulted in changes in the adrenal cortex as reflected in reduced aldosterone levels (66). This effect was suggested to be secondary to changes in the electrolyte or acid-base balance.

With regard to the possible significance of this lesion to humans, several factors must be considered: First, rats are more susceptible to adrenal medullary hyperplasia than are other species, including humans, and some scientists consider the lesion in rats to be irrelevant to humans (14, 27, 33, 101, 128-130). Second, the effects observed in the adrenal medulla in rats appear to be related in some way to the changes in calcium homeostasis. This link has not been observed in any other species, notably not in mice or humans. Third, the enhancing effects of slowly absorbed carbohydrates on calcium absorption are much more pronounced in rodents than in humans although, as noted above, the effects are observed in humans.

As a result the consensus is that the adrenal tumors in rats fed diets high in polyol or lactose have little or no significance for humans. Nevertheless, until the mechanism linking the polyol diets to adrenal medullary hyperplasia has been elucidated, the mechanism cannot definitively be ruled out in other species (see reviews 101). It should be noted, however, that the lesion has not been reported in any other species (including humans) at this time.

### *Effects Unique to One Polyol*

**EFFECT OF XYLITOL ON GASTRIC EMPTYING**—Several investigations have considered the effects of xylitol on gastric emptying and intestinal motility with regard to possible beneficial effects of xylitol on food intake. Whether other polyol sweeteners share these effects has not been determined, although the slowly absorbed sugar lactose has been noted to have similar effects (86, 97).

Salminen et al (141) showed that oral xylitol produced no change in the secretion of the gastric inhibitory polypeptide in rats, whether or not the animals had been adapted to xylitol. This effect was also observed in humans (141). Another study with rats showed that adaptation to xylitol resulted in a decrease in the rate of gastric emptying but that no change in the levels of the gastric inhibitory polypeptide was involved (137). Subsequently Shafer et al (149) observed that ingestion of 25 g of xylitol by humans led to decreased rates of gastric emptying and food intake during a subsequent meal, an effect not observed with glucose or fructose. Salminen et al (138) have since reported an increase in motilin secretion accompanying the increase in intestinal transit and the delay in gastric emptying.

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**XYLITOL AND OXALATE** Interest in the metabolic interrelationships between xylitol and oxalate arises from clinical observations of oxalate deposition in certain tissues of some patients infused with xylitol (36, 44, 146, 158) and reports of oxalate stones in laboratory rodents fed diets with high levels of xylitol (48, 49, 81). Subsequent investigations have noted oxalate formation during infusions or injections of xylitol in rats (68, 72, 131, 133) and humans (36, 118). The effect was not observed in similar studies with rabbits (120, 168) nor in all studies with humans (31, 36, 71, 169) or rats (70), although in some instances increases in other two-carbon acids were observed (31, 68, 70, 71, 133). Feeding studies have also given variable results. Some studies in rats (60, 140) and mice (17) showed no increases in oxalate. On the other hand, Bär showed increases in oxalate and glycolate excretion in mice fed xylitol-containing diets (10). In humans, slight increases in urinary glycolate but only occasional marginally significant increases in urinary oxalate occurred in response to xylitol (17); both compounds were labelled in the urine of individuals consuming labelled xylitol but not labelled glucose (15). The synthesis of oxalate in isolated liver cells (119, 132, 133) and liver tissue (69) was also observed, although the effect was not always specific for xylitol, and oxalate was also formed from other carbohydrates or polyols (119, 132). The most interesting observations in this regard were reported by Hauschildt & Brand (69), who did not observe oxalate synthesis from either glucose or xylitol under normal conditions but did observe oxalate formation in liver homogenates from both when substrate oxidation was enhanced. Under these conditions xylitol was 1.6 times as effective as glucose.

Initially, investigators of the metabolic relationship between xylitol and oxalate proposed the formation of glycolaldehyde, an oxalate precursor, by the release of "active glycolaldehyde" from the transketolase reaction (31, 72, 158). More recently, a direct pathway for the formation of oxalate from xylitol was proposed and investigated simultaneously by two independent laboratories (19, 84). This pathway involves the phosphorylation of the first metabolic product of xylitol oxidation, D-xylulose, by fructokinase to give xylulose-1-phosphate rather than by xylulokinase to give xylulose-5-phosphate. The xylulose-1-phosphate is subsequently cleaved by aldolase to give dihydroxyacetone-phosphate and oxalate precursor glycolaldehyde. Glycolaldehyde is converted to oxalate by way of glycolate and glyoxalate sequentially. Barngrover et al (19) devised enzymatic assays for xylulose-1-phosphate and glycolaldehyde and demonstrated the formation of both compounds in rat hepatocytes treated with D-xylulose. At the same time James et al (84) demonstrated the formation of xylulose-1-phosphate and glycolaldehyde in a reconstructed system using human liver fructokinase and aldolase.

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Subsequent studies in both laboratories have been aimed at quantifying this pathway and evaluating what factors might influence the formation and subsequent metabolism of the glycolaldehyde produced. Barngrover & Dills substantiated the role of fructokinase in the formation of xylulose-1-phosphate and glycolaldehyde in xylulose-treated hepatocytes but could not measure the formation of either compound in xylitol-treated hepatocytes, probably because of the insensitivity of the assay method devised (18). Similar difficulties were encountered in similar studies with labelled xylitols of high specific activities (40), although the authors did postulate an upper limit to the flux through the xylulose-1-phosphate pathway. They proposed that under their incubation conditions the flux of xylitol through the minor pathway as outlined could not exceed 5% of that of the overall rate of xylitol metabolism, corresponding to a conversion of less than 2% of the xylitol-carbon to two-carbon fragments (40).

James et al (85) extended their earlier studies to include fructokinase and aldolase from various tissues in several species and commented on the potentialities of the xylulose-1-phosphate pathway in several animal models. Bais et al (6) purified the human liver fructokinase to homogeneity and discussed its relationship to oxalate formation in human liver. They concluded that the flux through this pathway is generally minor but that it may contribute to oxalate formation. They subsequently reported extensive studies on inhibitory effects of various substances, including intermediates of oxalate metabolism on aldolase and fructokinase (7).

Dills & Audet (39) recently used a kinetic computer model to simulate flux through the metabolic pathways involving the two xylulose phosphates. To this end they purified and studied the xylulokinase from bovine liver to determine the kinetic parameters of its interaction with D-xylulose. Using these and other literature kinetic and enzyme level data concerning L-iditol dehydrogenase, fructokinase, xylulokinase, and aldolase, they calculated the potential fluxes and evaluated the factors influencing these fluxes. The formation of both xylulose-1-phosphate and glycolaldehyde was found to be potentiated by factors that increase the flux through the dehydrogenase step, notably any increase in the  $\text{NAD}^+/\text{NADH}$  ratio, and by any decrease in xylulokinase activity or any increase in fructokinase activity (39). The increase in the potential for glycolaldehyde formation with regard to an increase in xylitol oxidation is particularly interesting when considered with the previously mentioned differential stimulation of oxalate formation from xylitol by substrate oxidation increases reported by Hauschildt & Brand (69).

The significance of the minor pathway in humans remains unclear. Evidence for its existence is indirect and consists of the observation that urinary glycolate and oxalate excretion increase in volunteers consuming xylitol (11)

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and that labelled xylitol but not labelled glucose is converted to labelled glycolate and oxalate (15). At the most, the net conversion of xylitol carbon to oxalate appears to be about 0.5% experimentally (36) and less than 5% in the most unfavorable circumstances, i.e. when low levels of xylulokinase are assumed for humans, predicted by the kinetic computer model (39). The major metabolic precursors of oxalate, glycolate, and glyoxalate are known to have several metabolic precursors besides xylitol and several metabolic fates besides oxalate (see reviews 7, 76, 126). It has been suggested that the conversion of xylitol to oxalate is regulated primarily at the level of glyoxalate or glycolate metabolism (7, 15). Further work on the kinetics of the conversion of xylitol carbon to oxalate are needed, particularly with regard to the kinetics of human xylulokinase and alternative metabolic fates of glycolaldehyde, glycolate, and glyoxalate.

**BLADDER STONES, BLADDER TUMORS, AND XYLITOL** Mice consuming diets with high levels of xylitol exhibited an increased incidence of bladder calculi and an increased incidence of hyperplastic and neoplastic changes in the bladder (47, 48, 81). Evidence indicates that urinary stone formation is a reasonable explanation for the occurrence of bladder tumors in mice fed xylitol (see review 101). In mice (10, 101 and references therein) but not rats (140), xylitol increases oxalate excretion as noted above, whether by way of xylitol metabolism, increased oxalate absorption, or both, and it increases excretion of calcium in rodents. In mice, the consumption of xylitol therefore increases two of the urinary parameters that are linked to possible stone formation.

As noted above humans fed xylitol do show slightly higher-than-average oxalate excretions (10, 15); far greater changes are noted in glycolate excretion, however (10). In this regard, Conyers et al (35) examined the kinetics of oxalate formation from xylitol using a one-compartment model; they concluded that even small conversions, 0.5% in their model, may at times be significant during infusions. Their model can be extended to dietary xylitol if one assumes that the highest doses of xylitol consumed during the Turku sugar studies (145) were consumed over a 12-hour period in any given day. The largest long-term dosage of 100 g/day for 100 days and the largest one-time dosage of 400 g would be the equivalent of infusions of 0.80 and 3.20 mmol/h/kg body wt for an average subject weighing 70 kg. These levels would not raise levels of oxalate above the 0.10 mM level suggested to be the threshold for crystallization (35). This model does not, however, take into account any differences in enzyme or effector levels or any other sources of oxalate besides xylitol, whether they be metabolic or dietary. The polyol-induced increases in calcium absorption in humans (34, 176) are also not considered. With regard to the significance of the lesions noted in mice to

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humans, it should be noted that the occurrence of bladder or kidney stones in humans consuming xylitol has not been reported, despite numerous closely monitored, long-term studies (see previous sections and reviews). Nevertheless, certain subgroups still may exist within the general population, individuals susceptible to calcium oxalate stone formation for instance, who may be at risk if consuming large amounts of xylitol.

**LACTITOL AND LEYDIG CELL TUMORS IN RATS** Male rats fed diets containing high levels of lactitol or lactose were reported to have an increased incidence of Leydig cell tumors (151). These tumors are fairly common in the rat unlike in mice or humans (see review 101). In contrast, many earlier studies with lactose and a few with lactitol never demonstrated an increase in rat Leydig cell tumors. There is no ready mechanistic explanation as to why both lactose and lactitol would result in such tumors. A mechanism would almost certainly have to involve a common metabolite, galactose for instance, or a common physiologic change, such as the calcium absorption discussed previously. Neither of these has been reported associated with Leydig cell tumors. Considering the high consumption of lactose by humans and the extremely low incidence of human Leydig cell tumors, the results, even if reproducible, are of questionable significance for humans.

## SUMMARY AND CONCLUSIONS

The polyols are a family of bulk sweeteners, some of which are currently used in the United States and in other nations. The use of these compounds is likely to increase in the future.

The greatest advantage of polyols as sweeteners is their reduced cariogenicity compared with sucrose, fructose, or glucose. This reduced cariogenicity has been observed with all of the polyols considered in this review. Furthermore, evidence suggests that one of these polyols, xylitol, may have cariostatic properties. More research is needed to clarify the mechanism of this cariostatic effect.

Evidence suggests that moderate usage of the polyols in human diets over long periods is not likely to produce many toxic effects. This conclusion is supported by the facts that (a) both sorbitol and mannitol have been used as sweeteners for some time without apparent side effects, and (b) extensive long-term studies with dietary xylitol in Europe have not yielded any reports of toxicity. At this point there is no reason to believe that the disaccharide polyols differ significantly in a qualitative sense from sorbitol or mannitol with regard to their effects in humans.

There are some research needs with regard to the inclusion of the polyol sweeteners in human diets:

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1. All of the polyols can cause osmotic diarrhea in humans if higher levels are consumed. This fact is noted in the labelling of products containing mannitol and sorbitol in the United States (see "Current Regulatory Status"). If the disaccharide polyols are to be used as bulk sweeteners, further studies of the dose levels that can cause diarrhea may be needed.

2. The polyols, like other slowly absorbed carbohydrates, enhance the absorption of certain minerals, particularly divalent cations. More comparative and mechanistic studies of this effect are needed.

3. All of the polyols, lactose, and other slowly absorbed carbohydrates appear to cause adrenal medullary hyperplasia at high doses in laboratory rats. Evidence suggests that these lesions are linked in some way to the lactose or polyol-induced changes in calcium homeostasis. Despite long-term use of lactose, sorbitol, and mannitol in human diets, similar lesions in humans have not been reported and some investigators have concluded that the lesion in rats has no relevance to humans. Nevertheless further studies are needed to elucidate the mechanisms of the dietary lactose and polyol-induced adrenal hyperplasias in rats to ascertain definitively if they also operate in other species.

4. For xylitol alone among the polyols, the occurrence of calcium oxalate bladder stones and accompanying neoplastic lesions was noted in mice. While the available evidence strongly suggests that dietary xylitol will not be a major risk factor for stone formation in the general population, certain questions remain. A minor metabolic pathway exists that can give rise to the metabolic production of oxalate from xylitol. Further work is needed on this pathway and on the metabolism of oxalate precursors in general. In addition the possibility that subpopulations of humans might face an increased risk of calcium oxalate stone formation by high levels of dietary xylitol needs to be addressed.

5. A single study that reported an increased incidence of Leydig cell tumors in male rats fed high levels of lactitol or lactose needs to be repeated. If the effect were reproduced, additional studies on the mechanism would be in order. The probable significance of the effect with respect to humans is unknown but is likely to be important.

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## Low Digestible Carbohydrates (Polyols and Lactose): Significance of Adrenal Medullary Proliferative Lesions in the Rat

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### INTRODUCTION

#### *Background and Explanation*

Polyols<sup>2</sup> are used in the food industry, mainly as bulk sweetening agents, particularly as sugar replacements. Commercially important polyols include the monosaccharide-derived polyols mannitol, sorbitol, xylitol, and the disaccharide-derived forms maltitol, isomalt, and lactitol. Also of commercial importance are the polysaccharide-derived polyol mixtures such as maltitol syrup, formerly referred to as hydrogenated glucose syrup. Maltitol syrup, characterized by the presence of maltitol as the major component [50–98% as per Joint FAO/WHO Expert Committee on Food Additives (JECFA) specifications] represents one in a family of related polyols commonly referred to as hydrogenated starch hydrolysates (HSH). Other members of that family include sorbitol syrup ( $\geq 50\%$  sorbitol, anhydrous basis) and similar mixtures commonly referred to as HSH (not currently monographed) that are characterized by the presence of polysaccharide-derived polyols as the major component. From a toxicological perspective, there is no reason to believe that the toxicological profile of HSH products, such as sorbitol syrup, is substantively different than that of the oligo-/polysaccharide-derived components of maltitol syrup.

The results of long-term toxicity studies in rats and other species were the subject of a number of meetings by JECFA. The following is a review of the evaluations of the individual polyols by JECFA with emphasis on the proliferative lesions of the rat adrenal medulla.

**Sorbitol.** The first polyol to be reviewed by JECFA was sorbitol, which was considered at the 7th meeting of JECFA in 1963 (JECFA, 1964). At that time, JECFA granted sorbitol an unconditioned acceptable

daily intake (ADI) of 0–150 mg/kg body wt based on the results of metabolic studies of safe use as a parenteral in man and the results of a 17-month feeding study in rats showing no deleterious effects. An ADI of “not limited” was also granted conditional on the provision of additional data from lifetime animal studies. The unconditional ADI was changed to “unlimited” at the 9th meeting of JECFA (JECFA, 1967) and reaffirmed at the 17th meeting of JECFA (JECFA, 1974a). In 1978, a temporary ADI “not specified” was allocated to sorbitol at the 22nd meeting of JECFA (JECFA, 1978a) following review of a study in which adrenal medullary hyperplasia occurred in rats exposed to high dietary concentrations (20%) for a period of 2 years. An unconditional ADI not specified was allocated at the 24th meeting as a result of JECFA’s view that gross dietary and probable metabolic imbalance and stress were possibly causally related to the medullary lesions (JECFA, 1982).

**Mannitol.** Mannitol was first evaluated at the 10th meeting of JECFA (JECFA, 1967). Although at the time the data were less extensive for mannitol than for sorbitol, an unconditional ADI of 0–50 mg/kg body wt and an ADI of 50–100 mg/kg body wt conditional on the provision of additional long-term animal data were granted on the basis of metabolic studies. At the 18th meeting of JECFA (JECFA, 1974b), the ADI was changed to temporary status pending results of animal studies. The safety of mannitol was reevaluated at the 30th meeting of JECFA (JECFA, 1987a) when the results of one long-term rat feeding study, which had indicated an increase in the incidence of thymomas, and three additional studies in other strains of rats were reviewed. One strain of rat, the F344 rat, showed proliferative lesions of the adrenal medulla. After review, JECFA (1987a) changed the ADI to not specified in accordance with previous conclusions of the Expert Panel regarding the lack of relevance to humans of the adrenal lesions (JECFA, 1982).

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<sup>2</sup> For the purpose of this documentation, the term “polyols” is used for the sugar alcohols.



**Xylitol.** Xylitol was first evaluated at the 21st meeting of JECFA (JECFA, 1978b). At that time, JECFA not allocate an ADI due to clinical reports that there had been several deaths in human patients administered xylitol intravenously for parenteral alimentation. With the availability of additional animal toxicology data, xylitol was reevaluated by JECFA at the 22nd meeting (JECFA, 1978b). At that time, the issue of proliferative effects on the adrenal medulla was first raised due to results from a long-term feeding study in which xylitol was administered to rats at levels of up to 20% in the diet. In this study, sorbitol, which had been included as control, also produced an increased incidence of adrenal medullary hyperplasia. In a reevaluation of sorbitol at the 22nd meeting of JECFA (1978b), the committee concluded that the production of medullary hyperplasia in rats with 20% sorbitol was due to dietary imbalance, and possible metabolic imbalance, as well as physiological stress in the aging rat. No ADI was allocated to xylitol at the 22nd meeting of JECFA. At the 27th meeting of JECFA (JECFA, 1983), the subject of adrenal medullary hyperplasia and neoplasia associated with high dietary exposure of xylitol to rats was subjected to an additional review. At that time, it was noted that xylitol influenced calcium uptake and excretion in rodents. Based on the review of data for xylitol and the change in the ADI made for sorbitol, JECFA (1983) granted an ADI not specified for xylitol.

**Lactitol.** JECFA (1983) also evaluated lactitol at the 27th meeting, at the same time the reevaluation of xylitol was conducted. JECFA's (1983) review of the data on lactitol indicated that, as for xylitol, lactitol produced slight increases in the incidence of adrenal medullary hyperplasia and neoplasia in rats fed lactitol at a dietary concentration of 10% for a period of 2 years. Lactose, used as a control group, produced similar effects on the adrenal medulla at dietary levels of 20%. Effects on calcium homeostasis were also reported with lactitol and lactose. Based on these data and the data on the normal food ingredient lactose, as well as on extensive short-term animal and human data, JECFA (1983) allocated an ADI not specified to lactitol.

**Maltitol.** Maltitol, a disaccharide-derived polyol, was first evaluated as a separate entity by JECFA at the 33rd meeting (JECFA, 1989). Previously, material that contained 50–90% maltitol had been assessed as hydrogenated glucose syrup, now referred to as maltitol syrup. Other related polyol mixtures, also belonging to the family of polyol mixtures referred to as hydrogenated starch hydrolysates, may contain from 0 to less than 50% maltitol. The ADI not specified for hydrogenated glucose syrups containing 50–90% maltitol (now referred to as maltitol syrup) had been granted at the 29th JECFA meeting (JECFA, 1987b). This was based on extensive knowledge of metabolism and lack of obvi-

ous toxicity in experimental animal and human studies. When maltitol of greater than 98% purity was assessed as a separate food additive at the 33rd JECFA meeting (JECFA, 1989), the results of recently conducted metabolic studies on pure maltitol in conjunction with the toxicological database on maltitol syrup, including the data considered by JECFA in the granting of an ADI not specified for hydrogenated glucose syrups, were assessed to derive an ADI not specified for crystalline maltitol of greater than 98% purity.

At the 41st meeting of JECFA, results from a recent long-term toxicity/carcinogenicity study on a preparation containing 87% maltitol were reviewed (JECFA, 1993). In this study, in which groups of rats were administered the maltitol preparation in the diet at doses of 0, 0.5, 1.5, or 4.5 g/kg body wt/day (equivalent to about 0–10% in the diet), the total incidence of pheochromocytoma (benign and malignant combined) was increased only in the high-dose group. The number of malignant pheochromocytomas was increased in the low-dose group; however, the dose-response relationship was poor because no increase in the incidence of malignant pheochromocytoma was reported in the intermediate-dose group. The incidence of hyperplasia also showed a positive trend, although the actual incidence did not achieve statistical significance except in the high-dose females. Based on the known data from other polyols and earlier conclusions regarding the significance of the adrenal gland lesions, JECFA (1993) allocated an ADI not specified to maltitol.

**Maltitol syrup (hydrogenated glucose syrup).** Maltitol syrups, formerly referred to as hydrogenated glucose syrups (HGS), are a complex mixture of sorbitol, maltitol, and other related polysaccharide-derived polyols. Maltitol syrups contain 50–98% maltitol as specified by JECFA (1993). Maltitol syrups (hydrogenated glucose syrups) belong to the family of closely related polysaccharide-derived polyols commonly referred to as HSH. This family also includes other polysaccharide-derived polyol mixtures that contain less than 50% maltitol such as sorbitol syrups. Because the qualitative nature of the constituents of HSH, including sorbitol syrup, is the same as that of maltitol syrup, the toxicological profiles of HSH would be expected to be similar to that of maltitol syrups.

Hydrogenated glucose syrups, which contain 50–90% maltitol, were first assessed at the 24th meeting of JECFA, at which time JECFA concluded that there were insufficient data available for the preparation of a toxicological monograph, and hence no ADI specification was established (JECFA, 1980). At the 27th meeting of JECFA, an ADI of 0–25 mg/kg body wt was assigned to HGS on the basis of acute and subchronic toxicity tests, reproduction and metabolism studies in animals, and several human studies (JECFA, 1983). Following review of long-term toxicity studies demon-

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strating the low order of toxicity, JECFA (1987b) allocated an ADI not specified to HGS. JECFA renamed hydrogenated glucose syrups as maltitol syrup (JECFA, 1989), and extended the specifications to include products containing 90–98% maltitol (JECFA, 1993). At the 41st meeting, JECFA (1993) confirmed the ADI not specified for maltitol and for maltitol syrup that met the specifications for maltitol syrups established at that meeting (JECFA, 1993).

**Isomalt.** In 1981, at the 25th meeting of JECFA (1981), isomalt was evaluated. Based on the results of metabolic studies showing isomalt to be hydrolyzed to glucose, sorbitol, and mannitol, and short-term animal toxicity studies, JECFA (1981) allocated a temporary ADI of 0–25 mg/kg body wt pending the results of long-term animal toxicity tests that were in progress at the time. At the 29th meeting of JECFA (1987b), isomalt was reevaluated in light of the available long-term toxicity data. No toxic effects occurred in mice and rats exposed to isomalt for their lifetimes at levels of up to 10% in the diet. On the basis of the available data, JECFA (1987b) allocated an ADI not specified to isomalt.

**Summary of JECFA evaluations and future directions.** In summary, currently JECFA has allocated an ADI not specified for all of the polyols of commercial importance reviewed in this document. As indicated previously, all these polyols, except for isomalt, have been shown in long-term rat studies, at dietary levels of 10–20%, to induce hyperplasia and/or benign and malignant pheochromocytomas of the adrenal medulla. In light of the finding of adrenal lesions with a preparation containing 87% maltitol (discussed at the 41st meeting), JECFA (1993) proposed that the information database on medullary hyperplasia and pheochromocytomas associated with long-term studies in rats with polyols and other poorly absorbed carbohydrates be reviewed and that the mechanisms of the appearance and toxicological significance of these lesions be assessed at a future meeting. This paper has been prepared to assist JECFA in its review of the biological data regarding the occurrence of adrenal tumors in rats following polyol intake and the assessment of the relevance of these tumors to human consumption of polyols in foods.

The report first presents an overview of the long-term animal toxicology, human, and metabolism studies on polyols and lactose (Biological Data), following which the adrenal medullary proliferative lesions associated with high-dose dietary consumption of certain polyols in rats are discussed in terms of the species specificity of the response of the adrenal medulla and the mode of action involved in lesion development (Relevance to Humans of Rat Adrenal Medullary Proliferative Lesions). Based on this analysis, the relevance to humans of the rat adrenal medullary lesions reported in long-term toxicity studies conducted on certain poly-

ols is assessed (Summary and Conclusion). Additional details regarding the long-term animal toxicity tests, human studies, metabolism studies, and the evaluation of the species specificity of the response of the adrenal medulla are provided in Appendices A, B, C, and D, respectively.

## BIOLOGICAL DATA

The following section presents an overview of the results of mutagenicity/genotoxicity data, animal toxicology studies, and human tolerance and safety studies, which are pertinent to the assessment of the relevance to humans of adrenal medullary proliferative lesions reported in some of the long-term rat studies on certain polyols.

### Mutagenicity/Genotoxicity

All the polyols reviewed, including sorbitol, mannitol, xylitol, lactitol, maltitol, maltitol syrup, isomalt, as well as lactose, have been demonstrated to be nonmutagenic and nongenotoxic in a variety of *in vitro* test systems, including the Ames *Salmonella typhimurium* assay, both with and without the presence of an exogenous metabolic activation system (NTP, 1982; JECFA, 1983; Takizawa and Hachiya, 1984; FASEB, 1986; JECFA, 1987a,b; Bär, 1987a, 1988). All the genotoxicity assays performed *in vivo*, including the mouse micronucleus assay, have also yielded negative results (NTP, 1982; JECFA, 1983; Takizawa and Hachiya, 1984; FASEB, 1986; JECFA, 1987a,b; Bär, 1987a). Recent data on lactitol show exclusively negative results for the Ames test and chromosomal aberration assays, as well as in an *in vivo* mouse micronucleus assay (Iwakura *et al.*, 1994). Based on the available information, it is concluded that the polyols considered in this report are nonmutagenic and nongenotoxic.

### Synopsis of the Long-Term Animal Toxicology Studies

The results of long-term, generally 2-year, studies conducted on sorbitol (Hunter *et al.*, 1978a; Bär, 1984a,b), xylitol (Hunter *et al.*, 1987a; Bär, 1984a,b, 1986), lactitol (Sinkeldam *et al.*, 1992), and a preparation containing 87% maltitol (Conz and Fumero, 1989; Conz and Maraschin, 1992), reviewed in detail in Appendix A, show that these polyols are associated with significant dose-related increases in the incidence of adrenal medullary tumors and total number of proliferative lesions in rats when administered in the diet at concentrations generally in the range of 10–20%. A significantly increased incidence of adrenal medullary hyperplasia was reported in male rats fed xylitol at dietary concentrations of 5% for 2 years (Hunter *et al.*, 1978a). The results of one long-term study in which mannitol was administered at 10% in the diet have also shown a weak proliferative response in the adrenal

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medulla of rats (Gongwer *et al.*, 1978), but not in mice (NTP, 1982). No significant effects on the adrenal medulla occur at dietary concentrations below 5%. Similar studies conducted on sorbitol, xylitol, and lactitol in mice (Hunter *et al.*, 1978b; Til *et al.*, 1992) and dogs (Heywood *et al.*, 1977, 1981; Til *et al.*, 1992) have shown no effects on the adrenal medulla. Long-term toxicity studies on isomalt, in which isomalt was administered at up to 10% of the diet for 128–130 weeks, have not demonstrated any proliferative effects on the adrenal medulla in rats (Smits-Van Prooije *et al.*, 1990), mice (Smits-Van Prooije *et al.*, 1990), or dogs (Hoffmann *et al.*, 1981).

The finding of adrenal lesions in rats following high-dose exposure to certain polyols is not unique. Lactose, another low-digestible carbohydrate, also produces adrenal medullary proliferative lesions in rats (Sinkeldam *et al.*, 1983, 1992; Woutersen, 1987; de Groot *et al.*, 1995), but not in mice (Til *et al.*, 1986). In the studies reporting proliferative effects on the adrenal medulla in rats in response to feeding of high dietary concentrations of certain polyols or lactose, there are generally increases in the frequency of adrenal medullary hyperplasia (Hunter *et al.*, 1978a; Gongwer *et al.*, 1978; Woutersen, 1987; Sinkeldam *et al.*, 1983, 1992), benign adrenal medullary tumors (Hunter *et al.*, 1978a; Bär, 1984a,b; Woutersen, 1987; Sinkeldam *et al.*, 1983, 1992), and/or in the frequency of the combined incidence of these lesions (Hunter *et al.*, 1978a; Bär, 1984a,b, 1986; Conz and Fumero, 1989; Conz and Maraschin, 1992).

Adrenal medullary tumors have been reported to develop only after a minimum of 63 weeks of treatment (with xylitol) (Bär, 1986). Hyperplastic lesions of the adrenal medullary tissue appear to precede the appearance of tumors, suggesting that the effects of polyols on the adrenal medulla progress from simple hyperplasia to tumors. The hyperplastic and neoplastic lesions associated in rats with high dietary concentrations of polyols are similar histopathologically to spontaneous forms of these lesions. With respect to the potential threshold at which significant hyperplastic/neoplastic effects occur in the rat adrenal medulla, it should be noted that none of the long-term rat studies show statistically significant, dose-related effects on the incidence of hyperplasia, the total number of adrenal medullary tumors, or the total number of adrenal medullary proliferative lesions at dietary concentrations below 5%.

Increased incidences of adrenal medullary hyper-/neoplasia have been reported to occur following long-term dietary exposure to polyols in several rat strains, including Sprague–Dawley (Hunter *et al.*, 1978a; Conz and Fumero, 1989; Conz and Maraschin, 1992), Wistar (Sinkeldam *et al.*, 1992), Fuellinsdorf–Albino (Wistar-derived) (Bär, 1984a,b, 1986), and the F344 rat (Gongwer *et al.*, 1978). Although polyol consumption has

been associated with adrenal medullary proliferative lesions in several rat strains, strains most prone to the development of spontaneous proliferative lesions of the adrenal medulla (e.g., F344 and Wistar) appear to be particularly susceptible to the effects of polyols. Male rats, regardless of the strain used, show a greater spontaneous incidence than females of adrenal medullary proliferative lesions and a greater incidence of these lesions following high-dose treatment with certain polyols and lactose.

In addition to the effects on the adrenal medulla, the feeding of high doses of polyols, as well as lactose, to rats generally also results in slight decreases in body weight, cecal enlargement, pelvic nephrocalcinosis, and hypercalciuria (Gongwer *et al.*, 1978; Hunter *et al.*, 1978a; Sinkeldam *et al.*, 1983, 1992; Bär, 1984a,b, 1986; Roe and Bär, 1985; FASEB, 1986; Woutersen, 1987; Dills, 1989; Conz and Fumero, 1989; Conz and Maraschin, 1992). The slightly reduced body weights, which would be expected given the lower caloric values of the polyol-containing diet compared to the control diets, and the cecal enlargement, possibly resulting from the increased amount of osmotically active substances in the cecum, are not considered to be adverse effects per se and are not considered toxicologically significant.

Hypercalciuria, which is observed in rats fed diets containing high concentrations of polyols, has been documented to be associated with the increased incidence of adrenal medullary hyper-/neoplasia (Bär, 1986). Hypercalciuria likely results from increased calcium absorption (Fournier *et al.*, 1967; Hämmäläinen *et al.*, 1985; Amman *et al.*, 1988; Goda *et al.*, 1992; Brommage *et al.*, 1993) probably due to changes in the membrane permeability of the intestinal epithelium brought about by the increased osmotic pressure of the intestinal contents (Pansu *et al.*, 1975; Hämmäläinen and Makinen, 1989; Langkilde *et al.*, 1994). Increased acid loading, which could result from the fermentation of polyols to short-chain volatile fatty acids in the lower gut, may also contribute to the observed hypercalciuria (de Groot *et al.*, 1995), although data to directly support this hypothesis are lacking.

Bär (1986) studied the effect of dietary calcium on the occurrence of adrenal lesions with xylitol treatment. In this particular study, groups of 75 male Fuellinsdorf–Albino (Wistar) rats were fed diets containing 20% xylitol and 0.4, 0.2, or 0.05% calcium as the carbonate salt. Controls received 0% xylitol and 0.4% calcium in the diet. Treatment was continued for a period of 63 weeks. At histopathological examination, the incidence of hyperplasia and tumors of the adrenal medulla was significantly increased in the xylitol treated animals given 0.2 and 0.4% dietary calcium. In addition, by Week 13/14 urinary calcium excretion was considerably greater in the xylitol-fed rats administered a diet containing more than 0.05% calcium. By Week 53/54, urinary excretion

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**TABLE 1**  
**Effects Reported with Polyols and Lactose in Long-Term Rat Studies**

Substance	Effect				
	Adrenal medulla hyper-/neoplasia	Cecal enlargement	Pelvic nephrocalcinosis	Hypercalciuria	Reduced weight gain
Sorbitol	+	+	+	+	+
Mannitol	±	+	+	+	+
Xylitol	+	+	+	+	+
Lactitol	+	+	+	+	+
Maltitol	+	+	+	+	+
Maltitol syrup <sup>a</sup>	±	+	ND <sup>b</sup>	ND	+
Isomalt	-	+	+	ND	+
Lactose	+	+	+	+	+

Note. Adapted from Bär (1994).

<sup>a</sup> Formerly referred to as hydrogenated glucose syrups (HGS), and including products containing 90–98% maltitol. Maltitol syrups are one of a family of related polysaccharide-derived polyols referred to as hydrogenated starch hydrolysates.

<sup>b</sup> No data.

of calcium was greater than that of controls in all groups of xylitol-fed rats. The results of this study indicate that the adrenal medullary tumors associated with the administration of high dietary concentration of certain polyols in long-term rat studies may be mediated by alterations in calcium homeostasis (Bär, 1986, 1987a,b, 1988; FASEB, 1986; Tischler and DeLellis, 1988b).

In summary, the chronic studies conducted in rats demonstrate that adrenal medullary tumors develop in response to high dietary concentrations of certain polyols only after long-term treatment and only at high doses (i.e., ≥5% of the diet). These lesions are not observed in long-term feeding studies in mice or dogs. Given the lack of effect of long-term, high-dose polyol consumption on the adrenal medulla of species other than the rat, it is likely that this response is a species-specific phenomenon, potentially due to alterations of calcium homeostasis. A summary of the effects of the individual polyols, and lactose, in long-term rat studies is provided in Table 1.

Details of the animal toxicity studies that are pertinent to this assessment are presented in Appendix A.

#### *Synopsis of Studies Conducted in Humans*

A number of human studies have been conducted on polyols that have assessed the safety, cariogenicity, caloric value, and metabolism of polyols in man (Itoya *et al.*, 1974; Keup and Putter, 1974; Putter and Spengler, 1975; Siebert *et al.*, 1975; Scheinin and Mäkinen, 1975; Doorenbos, 1977; Zaal and Ottenhof, 1977; Mäkinen and Virtanen, 1978; Tacquet and Devulder, 1978; Förster *et al.*, 1981; Mäkinen *et al.*, 1981; Atsui *et al.*, 1982; Kearsley *et al.*, 1982; Secchi *et al.*, 1982; Bär, 1983, 1984b, 1985; Bär and Lamm, 1986; FASEB, 1994).

The short- and long-term human studies that pertain to the safety assessment of sugar alcohols used as sweetening agents reveal that all the polyols under con-

sideration have a favorable safety history because consumption of these polyols in controlled studies by human volunteers, as well as by the public at large, has not been associated with any significant adverse effects. The human studies show that even relatively high doses of polyols, on the order of up to 100 g/day, consumed over long periods of time have no effect on parameters, which could suggest the potential for polyols to have adverse effects on energy metabolism, liver function, or mineral homeostasis (Itoya *et al.*, 1974; Mäkinen and Virtanen, 1978; Scheinin and Mäkinen, 1975; Tacquet and Devulder, 1978; Mäkinen *et al.*, 1981; Bär, 1983, 1984b, 1988; Bär and Lamm, 1986). For example, in two clinical studies designed to assess the cariogenicity of xylitol, in which male and female volunteers consumed between 70 and 100 g of xylitol per day for 2 to 5.3 years, there were no effects on urinary concentrations of calcium, catecholamine, magnesium, phosphate, bilirubin, serum amyloid p-component (SAP), and amino acids, and no effects on biochemical and hematological parameters related to lipid, carbohydrate, or energy metabolism were observed (Mäkinen and Virtanen, 1978; Scheinin and Mäkinen, 1975; Mäkinen *et al.*, 1981). Another 32-month clinical study conducted with 157 children for the purposes of evaluating cariogenicity (Bär and Lamm, 1986) also demonstrated no effects of xylitol consumption at rates of up to 20 g/day on parameters measuring hepatobiliary function. Shorter-term studies, on the order of several weeks to several months duration, also have shown that, as for xylitol, other polyols, including lactitol (Doorenbos, 1977; Zaal and Ottenhof, 1977), maltitol (Itoya *et al.*, 1974; Tacquet and Devulder, 1978), and isomalt (Keup and Putter, 1974; Putter and Spengler, 1975; Siebert *et al.*, 1975; Spengler, 1978; Spengler and Schmitz, 1979), do not produce adverse effects in humans, even at elevated rates of consumption.

In contrast to the increased calcium absorption in-

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ferred to occur in rodents based on the observation of hypercalciuria in a number of studies with different polyols, several human studies conducted with sorbitol, lactitol, xylitol, and hydrogenated starch hydrolysates (Lycasin) have shown that several polyols either inhibited calcium absorption or no changes occurred in the urinary calcium levels. For example, Griessen *et al.* (1989) found that a 15-g dose of lactitol decreased the fractional absorption of calcium in 20 male volunteers measured using the ratio of oral-administered  $^{45}\text{Ca}$  and intravenously administered  $^{47}\text{Ca}$ . Egger *et al.* (1989) found no changes in the urinary excretion of calcium following consumption of 20–40 g/day lactitol for 30 days by 12 volunteers. Similarly, Francis *et al.* (1986) found that the fractional absorption of radiolabeled calcium in 10 women given 10 g of sorbitol was reduced. Nguyen *et al.* (1993) orally administered 20 g of sorbitol, xylitol, Lycasin, Maltisorb, or glucose to 10 healthy volunteers for 5 days. Although sorbitol, Lycasin, and Maltisorb had no effect on urinary calcium, xylitol increased the urinary excretion of calcium. In contrast, Förster *et al.* (1981) fed 12 male and female volunteers 40–100 g of xylitol per day for 18 days and found no increases in plasma calcium or urinary calcium. Bär (1985) administered xylitol (1 g/kg body wt/day, split into four daily doses) in various foods to 12 male and female volunteers over 7 days. No significant differences in 24-hr calcium excretion occurred between subjects given xylitol and those given sucrose. Bär and Lamm (1986) also found no differences in urinary calcium excretion in 157 Polynesian school children consuming up to 20 g xylitol/day in a caries prevention study. Likewise, Mäkinen *et al.* (1981) reported no differences in urinary calcium levels in 41 male and female subjects who consumed xylitol (approximately 50 g/day) for 2 years compared to control groups consuming fructose or sucrose.

The findings in the available human studies provide no evidence to suggest that the adrenal medullary lesions reported in rats in association with chronic, extreme rates of consumption of certain polyols could potentially be relevant to humans. In particular, the general lack of any clear or consistent effects of polyol consumption in humans on urinary excretion of calcium or catecholamine suggests that the increased urinary calcium excretion and associated proliferative effects on the adrenal medullary tissue seen in rats is likely a species-specific phenomenon, occurring through a mechanism, or mechanisms, that are not operative in humans.

Additional details on the key long-term human studies are presented in Appendix B.

#### Metabolism Studies

The metabolism of polyols of commercial importance was evaluated to determine whether there may exist a

common mechanism by which many of these substances cause an increased incidence of adrenal medullary hyper-/neoplasia in rats. The absorption, distribution, and metabolism of the monosaccharide polyols, sorbitol, mannitol, and xylitol, as well as the disaccharide polyols, lactitol, maltitol, and isomalt, and the polysaccharide-derived maltitol syrups and other closely related HSH products containing less than 50% maltitol have been extensively reviewed (FASEB, 1986; Dills, 1989; Bär, 1990; Modderman, 1993; FASEB, 1994).

The monosaccharide-derived polyols, including sorbitol, mannitol, and xylitol, and the hydrolysis products (monosaccharides or monosaccharide alcohols), except for glucose, of lactitol, maltitol, isomalt, and maltitol syrup are all absorbed from the digestive tract by passive diffusion (FASEB, 1986, 1994; Dills, 1989; Bär, 1990), and, as a result, enter the circulation at slower rates than glucose which is actively transported across the intestinal mucosa (Dills, 1989). Given the lack of an active transport system, ingestion of large quantities of polyols can result in a considerable portion reaching the lower digestive tract (FASEB, 1986; Dills, 1989; Bär, 1990; Modderman, 1993) where they are fermented by gut microflora mainly to short-chain volatile fatty acids that are subsequently absorbed and utilized (FASEB, 1986; Bär, 1990). The disaccharide-derived polyols, such as lactitol, maltitol, and isomalt, and the dimeric and polymeric components of maltitol syrup and other closely related polysaccharide-derived polyols referred to as HSH (containing less than 50% pure maltitol) are poorly absorbed from the upper digestive tract due to the lack of appropriate or efficient hydrolyzing enzymes (Grupp and Siebert, 1978; Nilsson and Jagerstad, 1987; Harju, 1988a,b), as well as the lack of an active transport system. Lactitol is not hydrolyzed to a significant extent (Nilsson and Jagerstad, 1987; Harju, 1988a,b), whereas maltitol, and to a lesser degree isomalt, are partially hydrolyzed to glucose, sorbitol, and/or mannitol (JECFA, 1987b; Dills, 1989). The hydrolysis products are either absorbed (glucose) or partially absorbed, and then fermented in the lower gut (sorbitol and mannitol) (FASEB, 1986). The rates of intestinal absorption, metabolism, and the physiological disposition for each of the polyols are provided in Table 2.

Once absorbed, xylitol and sorbitol are metabolized in the liver with almost 100% of absorbed xylitol and the majority of absorbed sorbitol metabolized through normal glycolysis/gluconeogenesis pathways (Bässler, 1969; FASEB, 1978, 1986; Allison, 1979; Dills, 1989). Due to the hepatic metabolism of xylitol and sorbitol, negligible amounts are excreted in the urine (FASEB, 1994). Mannitol, which is absorbed from the intestine, is only to a small extent metabolized in the liver (FASEB, 1994), with most of the absorbed mannitol excreted unchanged in the urine. The disaccharide-de-

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**TABLE 2**  
**Absorption, Metabolism, and Physiological Disposition in Humans of Various Polyols and Lactose**

Polyol	Origin	Amount hydrolyzed	Amount absorbed	Amount metabolized	Fermented
Sorbitol	Monosaccharide derived	NA <sup>a</sup>	Incomplete (50%)	100% of absorbed	+
Mannitol	Monosaccharide derived	NA	Incomplete (25%)	Negligible	+
Xylitol	Monosaccharide derived	NA	Incomplete (25%)	100% of absorbed	+
Lactitol	Disaccharide derived	Negligible	Negligible	Negligible	+
Maltitol	Disaccharide derived	Partially	Incomplete (up to 40%)	100% of absorbed	+
Maltitol syrup	Oligosaccharide derived	Partially	Incomplete	100% of absorbed	+
Isomalt	Disaccharide derived	Partially	Incomplete (up to 20%)	100% of absorbed, except mannitol	+
Lactose	Disaccharide	Partially	Up to 100%; less in lactase-deficient subjects	Negligible	+

Note. Source: FASEB (1994)

<sup>a</sup> NA, not applicable.

rived polyols maltitol and isomalt are partially absorbed as a result of hydrolysis to glucose, sorbitol, and mannitol (Dills, 1989). Only negligible amounts of maltitol and isomalt are absorbed as such and excreted via the urine (JECFA, 1987b; FASEB, 1994). The non-absorbed portion of polyols that reaches the microbially colonized parts of the gut is completely fermented to short-chain fatty acids as are other nondigestible, poorly absorbed carbohydrates (Bär, 1990).

Of those polyols that are metabolized in the liver, all are metabolized to products that are normally produced endogenously via the glycolysis/gluconeogenesis pathways and that have no genotoxic activity. Furthermore, as discussed under Mutagenicity/Genotoxicity, none of the parent polyols shows genotoxic activity (JECFA, 1983, 1987a,b; FASEB, 1986; Bär, 1988). As a result, metabolism to genotoxic products cannot explain the presence of the adrenal medullary lesions reported in the long-term rat studies.

Several important differences in the absorption and metabolism of individual polyols have a direct bearing on the interpretation of toxicological studies. First, as shown in Table 2, there are large differences in the rates of digestion, apparent absorption, and metabolism of the various polyols. Second, there are major differences in the degree of hepatic metabolism of the polyols once they are absorbed. For example, sorbitol is metabolized to fructose and enters glycolysis as triose phosphate, whereas xylitol enters glycolysis as hexose phosphate through the pentose phosphate shunt. Also, nearly all the xylitol and sorbitol absorbed is metabolized, whereas only a small proportion of mannitol is metabolized. As a result, there is no common metabolic pathway that could potentially link all the polyols to a direct action on the adrenal medulla. Considering that most of the polyols have effects on the adrenal medulla in rats, it is unlikely that these effects are the result

of a direct action of polyols or their metabolites on the adrenal.

It has been suggested that the fermentation of polyols and other poorly digested carbohydrates to short-chain volatile fatty acids in the lower gut could result in an increased acid loading to the body, which in turn could result in increased urinary excretion of cations such as calcium and indirectly influence the adrenal medulla via effects on calcium homeostasis (de Groot *et al.*, 1995). Although dietary acid/base loading has been demonstrated to modulate the incidence of adrenal medullary proliferative lesions in lactose-fed rats (de Groot *et al.*, 1995), the relevance of acid-loading, which could theoretically result from polyol fermentation in the lower gut to adrenal medullary lesion formation in the rat, is uncertain.

For a more detailed review of the metabolism of each polyol, please see Appendix C.

#### RELEVANCE TO HUMANS OF RAT ADRENAL MEDULLARY PROLIFERATIVE LESIONS

To determine the relevance to humans of the adrenal medullary proliferative lesions reported to occur in rats chronically fed high dietary concentrations of certain polyols, one must consider the context in which the lesions arise. Specifically, one must consider the species specificity of these lesions through a careful examination of the interspecies differences in the anatomy, physiology, and response to various stimuli of adrenal medullary tissue and the mode of action by which the tumors develop. Data from these evaluations, taken together with the empirical data derived from the animal and human studies conducted on polyols, underpin any conclusions made regarding the (non)relevance to humans of the rat adrenal medullary proliferative lesions reported in long-term rat studies on certain poly-

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ols. Since JECFA last evaluated the relevance to humans of the rat adrenal medullary lesions (JECFA, 1978a,b, 1982, 1993), several critical studies have been published that serve to elucidate the species specificity of the rat adrenal medullary tumors.

The following sections discuss the interspecies differences with respect to adrenal medullary anatomy, physiology, and response to various stimuli and then discuss the mode of action, involving altered calcium homeostasis, by which the adrenal lesions develop in the rat following chronic oral exposure to certain polyols.

#### *Adrenal Medullary Tissue: Interspecies Differences*

*Interspecies differences: Basic anatomy of the adrenal medulla.* Mammalian species all have paired adrenal glands located in the perirenal fat adjacent to the kidneys (Hamlin and Banas, 1990; Tischler and Coupland, 1994). The adrenals of humans and rodents are composed of two tissues, the cortex and the medulla, which are developmentally, histologically, and functionally unrelated. The cortex, which comprises up to 90% of the weight of the adrenal gland, is mesodermal in origin and serves in the production of adrenocortical steroid hormones such as glucocorticoids, cortisone, and some adrenal androgens and estrogens (Hamlin and Banas, 1990; Capen *et al.*, 1991). The principal glucocorticoid of the rat adrenal cortex is corticosterone (Yarrington and Johnston, 1994), whereas that of the human cortex is cortisol. The adrenal medulla of both rats and humans is derived from neuroectodermal precursors capable of differentiating into either endocrine cells or sympathetic neurons (Anderson, 1993). The secretory cells of the adrenal medulla, known as chromaffin cells, produce catecholamines, important in responses to stress, and a variety of neuropeptides with diverse functions (Tischler and DeLellis, 1988a; Hamlin and Banas, 1990; Capen *et al.*, 1991). The adrenal medulla is richly innervated by cholinergic preganglionic sympathetic nerve endings and by a variety of peptidergic endings that together regulate chromaffin cell function (Parker *et al.*, 1993).

Despite the general similarities of the anatomy of the adrenal medulla in rats and humans, several potentially important differences exist. First, in the rat adrenal medulla there exist two distinct chromaffin cell populations producing predominantly epinephrine (E) or norepinephrine (NE). These cell types were initially distinguished by the ultrastructural appearances of their secretory granules, with NE cell granules appearing electron dense and E cell granules appearing less electron dense and more finely particulate (Coupland, 1969, 1989). A number of functional differences have been described, including differences in expression of cell adhesion molecules (Léon *et al.*, 1992), regulators of heterotrimeric G proteins (Vitale *et al.*, 1995),

and "SNAP" proteins involved in docking of secretory granules for exocytosis (Kannan *et al.*, 1996). E and NE cells also differ in their sensitivity to  $\text{Ca}^{2+}$  (calcium ion) as a mediator of exocytosis (Powis and Clark, 1995). In humans, there are no clear distinctions between chromaffin cell types (Brown *et al.*, 1971; Tischler and DeLellis, 1988a; Capen *et al.*, 1991; Tischler and Coupland, 1994) because E and NE are stored in a single cell (Capen *et al.*, 1991). Second, in the rat adrenal medulla, there is a third cell type, namely the small granule containing (SGC) cell (a cell whose functions remains largely unknown), for which there is no clearly defined human counterpart (Coupland *et al.*, 1977; Coupland, 1989; Tomlinson and Coupland, 1990; Capen *et al.*, 1991; Tischler and Coupland, 1994). Finally, the peptide and protein composition of secretory granules differs between rats and humans. For example, the granules in rats are composed largely of chromogranin A (Fischer-Colbrie and Frischenschlager, 1985), whereas those in humans are composed of equal proportions of chromogranins A and B (Schober *et al.*, 1987). These proteins, which are the major components of secretory granules by weight (Fischer-Colbrie and Frischenschlager, 1985; Winkler *et al.*, 1986; Schober *et al.*, 1987), are able to serve as precursors to a number of biologically active peptides.

The significance of interspecies differences in the anatomy of adrenal medullary tissue is not clear because at the present time the impact of these differences on the function of the adrenal medullary tissue in rats and humans remains unknown. However, such differences do allude to potential differences in physiology—differences that could be involved in the development of adrenal medullary lesions in rats fed high dietary concentrations of certain polyols, an effect not duplicated in any other species, including humans. Documentation of the anatomical differences in the adrenal medulla of rats and humans is provided in Appendix D.

*Interspecies differences: Calcium signaling.* Calcium plays an important role in intracellular signaling and, through the alteration of the activity of protein kinases and adenylate cyclases, can directly influence gene expression and cell proliferation. As a result, interspecies differences in extra- and intracellular calcium mobilization/transport, as well as differences in sensitivity to changes in intracellular  $\text{Ca}^{2+}$ , concentrations could be important in the proliferative response of the adrenal medulla to agents that affect calcium metabolism.

In humans and rodents, the secretory chromaffin cells utilize  $\text{Ca}^{2+}$  from both extracellular fluid and intracellular stores as a second messenger for regulating numerous cell functions, including exocytosis, release of catecholamines, and potentially the regulation of cell proliferation. Extracellular  $\text{Ca}^{2+}$  may enter a chromaf-



fin cell following neural stimulation by either a nicotinic cholinergic receptor channel or a voltage-gated channel. Entry of extracellular  $\text{Ca}^{2+}$  into the chromaffin cell triggers the release of calcium into the cytosol from intracellular stores including secretory granules and endoplasmic reticulum via a channel known as the ryanodine receptor (Berridge, 1993; Clapham, 1995).  $\text{Ca}^{2+}$  may also enter the cytosol from intracellular stores by the action of inositol triphosphate generated as a consequence of muscarinic cholinergic receptor activation (Malhotra *et al.*, 1989). In turn, intracellular cytosolic  $\text{Ca}^{2+}$ , alone and/or bound to calmodulin, can trigger exocytosis and increase the activity of enzymes including kinases and adenylate cyclases, leading to changes in gene expression in chromaffin cells (Ghosh and Greenberg, 1995). Studies suggest that  $\text{Ca}^{2+}$  may affect different cellular functions depending on its route of entry into the cell (Ghosh and Greenberg, 1995; Gandia and Garcia, 1995).

Chromaffin cells appear to express several types of voltage-gated  $\text{Ca}^{2+}$  channels. Although the channels in human chromaffin cells have not yet been characterized, the distribution of channel types on rat chromaffin cells differs from the distribution on cells from several other species (Gandia and Garcia, 1995). Should there be differences between the voltage-gated  $\text{Ca}^{2+}$  channels in the chromaffin cells of rats and humans, they could be related to the sensitivity of the rat chromaffin cell to certain mitogens and cell signaling effectors (see Interspecies Differences: Response of the Adrenal Medulla to Mitogens). An additional, thus far distinctive characteristic of rat chromaffin cells is the presence of spontaneous oscillation in cytosolic  $\text{Ca}^{2+}$  *in vitro* (D'Andrea *et al.*, 1993). In summary, the oscillation of cytosolic  $\text{Ca}^{2+}$  concentrations reported to occur in the rat, but not in other species, along with possible differences between rats and other species in the voltage-gated channels that regulate entry into the chromaffin cell of extracellular  $\text{Ca}^{2+}$ , could potentially render rat chromaffin cells particularly sensitive to altered calcium homeostasis.

*Interspecies differences: Response of the adrenal medulla to mitogens.* Recent studies of chromaffin cell proliferation by Tischler and colleagues may be of particular importance in assessing the relevance to humans of adrenal medullary proliferative lesions reported in long-term rat studies following high-dose ingestion of certain polyols.

The numerous agents that induce pheochromocytomas in rats (see Interspecies Differences: Proliferative Lesions of the Adrenal Medulla) are pharmacologically diverse and, for the most part, nongenotoxic. It has been hypothesized that a common denominator in their mode of action is stimulation of chromaffin cell proliferation by neurally derived signals that also regulate catecholamine production and release. This mitogenic re-

sponse, which might provide a means for long-term adjustment of adrenal medullary responses to increased physiological demand, could also provide a backdrop for mutations or other genetic damage (Tischler *et al.*, 1989, 1993, 1994, 1995; Tischler and Riseberg, 1993). Evidence to support this hypothesis comes from studies showing that chromaffin cell proliferation in the adult rat adrenal medulla is robust throughout life (Tischler *et al.*, 1989), is markedly decreased in response to adrenal denervation (Tischler *et al.*, 1991), and is increased by short-term administration of reserpine (Tischler *et al.*, 1989, 1991), which is known to cause a reflex increase in splanchnic nerve activity (Sietzen *et al.*, 1987). Prolonged administration of reserpine leads to development of pheochromocytoma in the rat adrenal medulla (Diener, 1988).

Neuronal stimulation of chromaffin cells results in activation of both protein kinase C via acetylcholine binding to muscarinic cholinergic receptors (Malhotra *et al.*, 1989) and adenylate cyclase and protein kinase A via receptor binding of neuropeptides (Tischler *et al.*, 1985; Watanabe *et al.*, 1992). These second messenger systems cross-communicate and act at multiple levels to regulate calcium mobilization and subsequent exocytotic secretion of catecholamines (Malhotra *et al.*, 1989). In cell cultures, proliferation of adult rat chromaffin cells is stimulated both by the phorbol ester, phorbol myristate acetate (PMA), which mimics the effects of muscarinic cholinergic innervation by activating protein kinase C, and by forskolin or cholera toxin, which mimic peptidergic innervation by activating adenylate cyclase (Tischler *et al.*, 1994). Consistent with the latter finding, the neurotransmitter pituitary adenylate cyclase activating peptide (PACAP) has been reported to be directly mitogenic to rat chromaffin cells at physiological concentrations (Tischler *et al.*, 1995). The peptide growth factors, nerve growth factor (NGF), and fibroblast growth factor (FGF) are also mitogenic for adult rat chromaffin cells (Malhanthappa *et al.*, 1990; Tischler *et al.*, 1994). In contrast to the results obtained with *in vitro* rat chromaffin cell preparations, similar cell culture experiments have shown that chromaffin cells from humans are inherently less responsive to mitogenic agents (Tischler and Riseberg, 1993). Moreover, based on immunohistochemical studies of the endogenous proliferation antigens, proliferating cell nuclear antigen (PCNA) and MIB-1/Ki67, adult human chromaffin cells show very little proliferation *in vivo* (A. S. Tischler, unpublished results). A summary of the different responses of the rat and human adrenal medullary chromaffin cell to various mitogenic stimuli is presented in Table 3.

Because of their greater sensitivity to mitogenic stimuli, chromaffin cells of the rat appear not to represent an appropriate model to assess the potential effects of chemicals on human chromaffin cells. The high susceptibility of the rat chromaffin cell to mitogenic

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**TABLE 3**  
**Response of Rat and Human Chromaffin Cells**  
**to Mitogenic Stimuli *in Vitro***

Mitogenic agent and signaling effector	BrdU labeling (% of cells labeled)	
	Rat	Human
NGF (receptor tyrosine kinase)	Up to 40	<0.1
FGF (receptor tyrosine kinase)	Up to 40	<0.1
PMA (protein kinase C)	Up to 10	<0.1
Forskolin (adenylate cyclase)	Up to 40	<0.1

*Note.* The table summarizes BrdU labeling indices in chromaffin cell cultures maintained for 2–5 days with BrdU and the indicated mitogens. Data for rat cells are in references by Tischler *et al.* (1994). Data for responses of human cells to NGF and PMA are from Tischler and Riseberg (1993). Data for effects of FGF on human cells are from A. S. Tischler, unpublished results. PMA, phorbol myristate acetate; NGF, nerve growth factor; FGF, fibroblast growth factor; BrdU, bromodeoxyuridine.

stimulation likely in part accounts for the relatively high rate of spontaneous pheochromocytoma development in rats compared to humans (see Interspecies Differences: Proliferative Lesions of the Adrenal Medulla). The susceptibility of the rat to the development of adrenal medullary lesions, therefore, is likely to be species specific and dependent on genetic factors controlling responsiveness of the chromaffin cell to proliferation-inducing stimuli. A detailed analysis of the response of the rat and human chromaffin cells, and the mechanisms governing these responses, is presented in Appendix D.

*Interspecies differences: Proliferative lesions of the adrenal medulla.* There are several important differences between rats and humans in the proliferative lesions that develop in the adrenal medulla. These differences, which provide strong evidence to indicate that the response of the adrenal medulla of rats following high dose oral exposure to certain polyols is specific to the rat, are briefly summarized below. For more details, and for a general discussion of the anatomy and physiology of adrenal medullary proliferative lesions, see Appendix D.

The most notable difference between rats and humans is the frequency with which spontaneous lesions of the adrenal medulla develop. In the rat, the spontaneous incidence of proliferative lesions of the adrenal medulla, including diffuse and focal hyperplasia as well as pheochromocytomas, is considerably greater and shows much more variability than similar lesions in the normal human population (Cheng, 1980; Thompson *et al.*, 1981; Strandberg, 1983; Roe and Bär, 1985; Tischler *et al.*, 1985, 1989; Manger *et al.*, 1985; Tischler and DeLellis, 1988b; Hamlin and Banas, 1990; Duprat *et al.*, 1990; Capen *et al.*, 1991). The spontaneous inci-

dence rate of tumors of the adrenal medulla in rats ranges from 0.5% in the Holtzman rat strain (Schardain *et al.*, 1968) to 17% in the F344 rat (Sher *et al.*, 1982) and to 69% in the Wistar rat (Pollard and Luckert, 1989). In contrast, the spontaneous incidence rate in humans has been reported to range from 0.005 to 0.1% (Minno *et al.*, 1954; von Schlegel, 1960; Symington, 1969; Manger *et al.*, 1985), with most data favoring the lower part of this range. The difference between rats and humans in the occurrence of spontaneous lesions of the adrenal medulla may relate to the unique sensitivity of rat chromaffin cells to mitogenic stimuli (see Interspecies Differences: Response of the Adrenal Medulla to Mitogens) or to several morphological and functional differences between rat and human chromaffin cells (see Interspecies Differences: Basic Anatomy of the Adrenal Medulla). Interestingly, mice, like humans, have chromaffin cells resistant to *in vitro* mitogenic stimuli (Tischler and Riseberg, 1993). Mice also have a low spontaneous incidence of adrenal medullary lesions (about 1%) (Tischler and Sheldon, 1995).

A second important species difference is that pheochromocytomas are inducible in rats by many pharmacologically unrelated substances (Tischler *et al.*, 1989) but have never been reported to be associated with any substance in humans. Induction of pheochromocytomas in rats appears to reflect an exacerbation of their tendency for spontaneous development (Tischler *et al.*, 1989).

A third difference between the adrenal lesions reported to occur in rats and humans is that in rats these lesions are generally not hyperfunctional (i.e., do not induce symptoms of hypertension) (Thompson *et al.*, 1981; Bosland and Bär, 1984; Roe and Bär, 1985; FASEB, 1986; Tischler and DeLellis, 1988b). In humans, however, pheochromocytomas are often accompanied by symptoms indicative of hypertension (i.e., increased catecholamine output). Possibly related to this are reports that adrenal medullary tumors in rats appear to have little or no ability to synthesize epinephrine, whereas adrenal medullary tumors in humans often produce abundant epinephrine (Thompson *et al.*, 1981; Roe and Bär, 1985; FASEB, 1986; Tischler and DeLellis, 1988b; Tischler *et al.*, 1990).

The striking differences between rats and humans in the incidence of spontaneous and induced adrenal medullary lesions, along with the morphological and functional differences between the lesions themselves, strongly indicate that the adrenal medullary proliferative lesions reported in rats administered high dietary concentrations of certain polyols is a species-specific phenomenon, likely of no relevance to humans.

#### *Polyols and Adrenal Medullary Proliferative Lesions: Mode of Action*

Aside from the data indicating that the adrenal medullary proliferative lesions observed in certain of the

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TABLE 4  
Effects of Xylitol-Calcium on Incidence of Adrenal Medullary Proliferative Lesions in Rats

Group	Treatment	No. with animals	No. with hyperplasia (%)	No. with neoplasia (%)	Urinary calcium (mg/24 hr)		
					Week 13/14	Week 32/32	Week 53/54
Control	0% Xylitol/0.4% Ca	63	40 (64)	6 (9.5)	0.6	0.6	0.8
1	20% Xylitol/0.4% Ca	56	31 (55)	24 (43)	6.1	11.1	7.0
2	20% Xylitol/0.2% Ca	57	39 (68)	17 (30)	2.7	8.6	7.8
3	20% Xylitol/0.05% Ca	63	55 (87)	7 (11)	0.8	2.9	3.0

long-term rats studies on some of the sugar alcohols is a species-specific response, the results of several recent experiments provide additional evidence to that previously reviewed by JECFA—that the mode of action of the development of these lesions involves alterations to calcium homeostasis, a mode of action that is operational in rats but not in humans.

As presented under Biological Data, hypercalciuria, and to a lesser extent increased serum calcium concentrations most likely resulting from increased calcium absorption from the gut based on the results of mechanistic studies performed by Fournier *et al.* (1967), Hämmäläinen *et al.* (1985), Amman *et al.* (1988), Goda *et al.* (1992), and Brommage *et al.* (1993), occurs in the long-term rat studies conducted on most of the polyols as well as those conducted on lactose. Given these observations and previous reports associating lactose consumption in rats with increased serum calcium and increased catecholamine content of the adrenal medulla (Brion and Dupuis, 1980), Bär (1986) suggested the existence of a relationship in rats between polyol consumption and increased calcium absorption (inferred from observed hypercalciuria) and the development of adrenal medullary proliferative lesions.

Bär (1986) was first to demonstrate conclusively that calcium was at least indirectly involved in the development of adrenal medullary lesions in polyol- and lactose-fed rats. In Bär's (1986) study, Wistar-derived rats were fed diets containing 20% xylitol and 0.05, 0.2, or 0.4% calcium for up to 63 weeks. Controls received a diet containing 0.4% calcium but no xylitol. Results of this study are presented in Table 4.

As can be seen from Table 4, a reduction in dietary calcium levels led to a reduction in urinary calcium levels and significantly decreased the incidence of neoplasia observed. However, the total incidence of combined hyperplasia and neoplasia appears to remain the same.

In contrast to the increased absorption and urinary excretion of calcium found in a number of rat studies with different polyols, several human studies conducted with sorbitol, lactitol, xylitol, and maltitol syrup (Lycasin, hydrogenated glucose syrup) have shown that several polyols either inhibited calcium absorption or

produced no changes in the urinary calcium levels (Förster *et al.*, 1981; Mäkinen *et al.*, 1981; Bär, 1985; Bär and Lamm, 1986; Griessen *et al.*, 1989; Egger *et al.*, 1989; Nguyen *et al.*, 1993). The effect of lactose on human calcium absorption is controversial, with the results of several studies showing enhanced absorption, whereas the results of other studies showed no effects (Allen, 1982; Roe, 1989; Brink *et al.*, 1993). Even in studies with lactose in which increased absorption occurred, the increased calcium absorption in humans was relatively small compared to the large increases reported in rats (Cochet *et al.*, 1983). Similarly, the urinary calcium levels in a number of human studies have either shown no increases or in some cases decreases (Mills *et al.*, 1940; Greenwald *et al.*, 1963; Condon *et al.*, 1970; Kocian *et al.*, 1973; Brink *et al.*, 1993).

New data from Tischler *et al.* (1996, in press) have strengthened the hypothesis that altered calcium homeostasis is involved in the development of the rat adrenal medullary proliferative lesions in the long-term rat studies conducted on certain polyols and lactose. Tischler *et al.* (1996, in press) demonstrated that administration of either 20,000 or 40,000 IU of vitamin D<sub>3</sub> to groups of six male Sprague-Dawley rats resulted in dramatic increases in the serum calcium concentrations and concomitant increases in the frequency of labeled chromaffin cells as measured by bromodeoxyuridine incorporation at each week of the 4-week experimental period. For example, after 1 week of treatment, the percentage of chromaffin cells labeled in the control, 20,000 IU, and 40,000 IU treatment groups was approximately 5, 20, and 30%, respectively (see Fig. 1). These rates remained stable throughout the 4-week duration of the study. Serum calcium levels were significantly higher at all time intervals in the vitamin D<sub>3</sub>-treated rats. Vitamin D<sub>3</sub> itself was not responsible for the increased rates of cell proliferation because it showed no mitogenic effects on rat chromaffin cells in *in vitro* assays (Tischler *et al.*, 1996, in press). In parallel with the increased rates of cell proliferation observed in the vitamin D<sub>3</sub>-treated rats, the relative weights of the adrenal gland were increased significantly over controls at each of the 4 weeks in the 40,000 IU dose group and at Weeks 2, 3, and 4 in the 20,000

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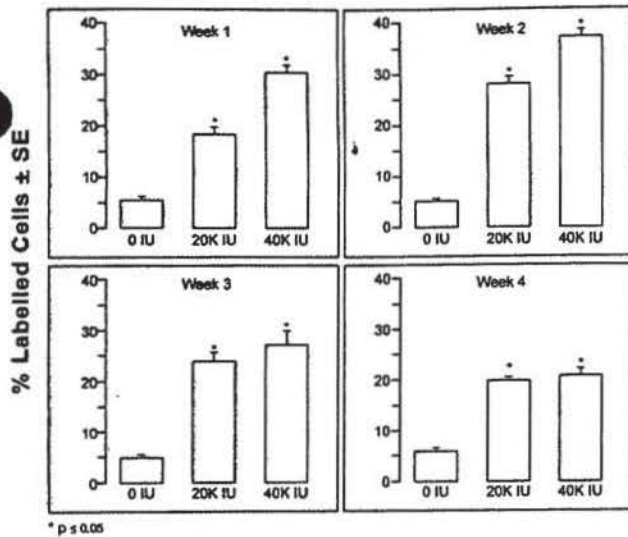


FIG. 1. Effects of vitamin D<sub>3</sub> on percentage of chromaffin cells labeled with BrdU during Weeks 1, 2, 3, or 4 of dietary supplementation. A 4-week study of the effect of dietary vitamin D<sub>3</sub> on chromaffin cell proliferation was conducted using adult male Charles River Crl:CD BR rats (Charles River Laboratories, Inc., Raleigh, NC) approximately 10 weeks of age at the beginning of treatment. Rats were acclimated for approximately 2 weeks prior to the start of the experiment. Rats treated with vitamin D<sub>3</sub> (Sigma, Cat. No. C9756) received 20,000 or 40,000 IU of vitamin D<sub>3</sub>/kg/day administered in corn oil via oral gavage once daily in a dose volume of 5 ml/kg/day. Control rats received corn oil alone. One week prior to sacrifice, rats were anesthetized with ether and Alzet osmotic pumps (Alza Corp, Palo Alto, CA, Model No. 2004) containing 20 mg/ml of 5-bromo-2'-deoxyuridine (BrdU; Sigma Chemical Co., St. Louis, MO, Cat. No. 5002) were implanted intraperitoneally. \*Indicates statistically significant increase over corn oil controls. Bars represent mean  $\pm$  SE for six rats per group. At least 500 chromaffin cells were scored for each rat using an ocular grid. From A. S. Tischler *et al.* (1996, in press).

IU dose group (Tischler *et al.*, 1996, in press). This increase is particularly dramatic given that the adrenal medulla comprises only 10% of the normal adrenal weight (Hamlin and Banas, 1990). Tischler *et al.* (1996, in press) also conducted similar experiments on xylitol and lactose. In these experiments, groups of six rats were treated with starch (controls), 20% lactose, or 20% xylitol in the diet for 4 weeks. Although no effects of xylitol or lactose on relative adrenal weights were recorded, a slight but significant increase in the percentage of labeled chromaffin cells was found in the lactose- and xylitol-treated rats at Weeks 2, 3, and 4 (see Fig. 2). This followed an initial slight but not significant decrease at Week 1. Based on these new data, and the knowledge that polyol consumption by rats increases calcium absorption, it must be concluded that increased calcium absorption is intimately involved in the genesis of the adrenal medullary lesions reported in the long-term rat studies conducted on certain polyols and on lactose.

The new data provided by Tischler *et al.* (1996, in

press), along with the classic study of Bär (1986), suggest that increased calcium absorption, as inferred from measured hypercalciuria in the long-term rat studies on certain polyols and from data derived from mechanistic studies on calcium absorption (Fournier *et al.*, 1967; Hämläinen *et al.*, 1985; Amman *et al.*, 1988; Goda *et al.*, 1992; Brommage *et al.*, 1993), is involved in the stimulation of adrenal chromaffin cell proliferation and likely to the subsequent development of neoplasia (Hunter *et al.*, 1978a; Saatman *et al.*, 1978; Bär, 1984a, 1986; Woutersen, 1987; Sinkeldam *et al.*, 1992). This would seem especially likely because the rat is uniquely sensitive to the development of these adrenal medullary proliferative lesions (see Appendix D). Data available on other chemicals indicate that retinol acetate (vitamin A) (Kurokawa *et al.*, 1985) and certain nonsteroidal anti-inflammatory agents, particularly zomepirac sodium (Mosher and Kircher, 1988), also appear to produce proliferative lesions in the adrenal medulla of rats via a mechanism involving increased calcium absorption.

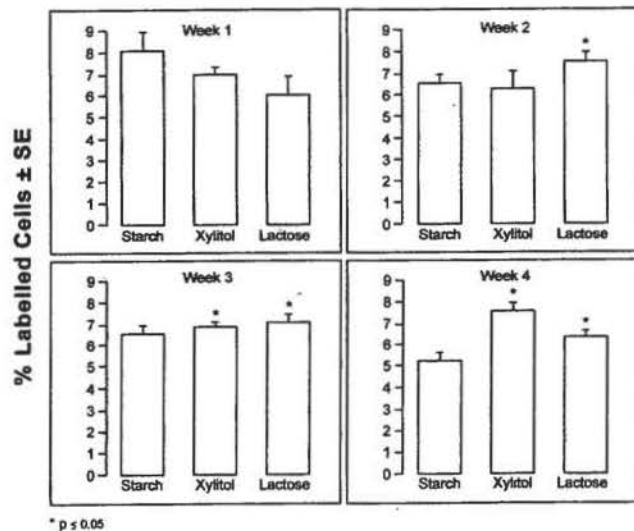


FIG. 2. Effects of xylitol and lactose on percentage of chromaffin cells labeled with BrdU during Weeks 1, 2, 3, or 4 of dietary supplementation. A 4-week study of dietary lactose and xylitol on chromaffin cell proliferation was conducted concurrently with the studies of vitamin D<sub>3</sub>, described in Fig. 1, using adult male Charles River Crl:CD BR rats (Charles River Laboratories, Inc., Raleigh, NC) approximately 10 weeks of age at the beginning of treatment. Rats were acclimated for approximately 2 weeks prior to the start of the experiment. Lactose (Sigma Chemical Co., St. Louis, MO, Cat. No. L3625) and xylitol (Sigma, Cat. No. X3375) were administered to rats via dietary admixtures containing 20% lactose or xylitol in standard rodent chow (Purina Certified Rodent Chow 5002, meal). Animals in the xylitol group were acclimated to xylitol during the first week with 10% dietary xylitol, and the concentration was increased to 20% for the second, third, and fourth weeks. Control animals received a diet isocaloric to the supplemented diets containing 20% cornstarch (Sigma, Cat. No. S4126). \*Indicates statistically significant increase over cornstarch controls. Bars represent mean  $\pm$  SE for six rats per group. At least 500 chromaffin cells were scored for each rat using an ocular grid. From A. S. Tischler *et al.* (1996, in press).

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Because hypercalciuria (inferred to indicate increased absorption) occurs in a dose-related manner in the rat, but not to any significant extent in humans ingesting up to 100 g of individual polyols per day (Mäkinen *et al.*, 1981), and because altered calcium homeostasis (a phenomenon that predisposes to adrenal medullary tumor development in rats) does not appear to operate in humans ingesting dietary amounts of polyols, the rat adrenal medullary lesions appear, on mechanistic grounds alone, to be irrelevant to humans.

#### SUMMARY AND CONCLUSION

In summary, many of the polyols in commercial use, as well as lactose, have been reported to be associated with increased incidences of adrenal medullary proliferative lesions in rats following long-term administration in the diet at concentrations of at least 5% and usually of 10–20%. At similar concentrations, polyols show no effects on the adrenal medulla of mice or dogs. In the rat studies, associated with the adrenal medullary lesions are hypercalciuria, cecal enlargement, and slight reduction in body weights. These observations are of no toxicological concern because they relate to the poor absorption and reduced caloric value of these compounds. The results of a number of human studies show that polyols are well tolerated, show no toxic effects, and have a safe history of use even at consumption levels of up to 100 g per day.

A critical review of the animal toxicology, human, and metabolism data shows that the adrenal medullary lesions seen in the rats cannot be explained on the basis of potential metabolic differences *per se* of absorbed polyols (i.e., systemic levels of polyols) between rats and humans. Furthermore, polyols and lactose are non-genotoxic and are not metabolized to genotoxic products. As a result, the relevance to humans of the adrenal medullary lesions reported in some of the long-term rat studies was evaluated on the basis of previously reviewed and new data regarding the species specificity of the response of the rat adrenal medulla and the potential mode of action by which the adrenal medullary proliferative lesions might arise. Based on these data, presented and discussed in the preceding sections and in Appendices A–D, it has been concluded that the adrenal medullary lesions reported to occur in rats following exposure to certain polyols are a species-specific phenomenon, active only at high doses and which occurs via a mode of action operative in rats but not in humans. The highlights of the data supporting this conclusion include

- The lack of genotoxic activity of parent compounds and metabolites
- The lack of any effect of polyols or lactose on the adrenal medulla of mice or dogs
- The development of adrenal medullary lesions in the rat occurs only at doses of 5% or more in the diet

- The lack of any toxicity of polyols to humans and the safe history of use of these compounds in a variety of food products

- Important interspecies differences with respect to morphology, physiology, and functionality of normal adrenal medullary tissue and of adrenal medullary proliferative lesions

Important interspecies differences in the response of the adrenal gland to mitogenic stimuli, with the rat being extremely sensitive and humans and mice relatively resistant

The extreme susceptibility of the rat to the spontaneous development of proliferative lesions of the adrenal medulla

Tumors are inducible in the rat adrenal gland, whereas there are no known inducers of human adrenal medullary tumors

- Strong evidence implicating increased calcium absorption as the mode of action by which the adrenal tumors develop in rats in response to high-dose polyol administration, a mode of action that is not operative in humans.

Based on the species specificity of the response of the rat adrenal medulla and the species-specific nature of the mode of action, the adrenal medullary lesions reported in the long-term rat studies on certain polyols have no relevance to humans.

With the conclusion that the adrenal medullary lesions are of no relevance to humans, the conclusions reached by the most recent JECFA evaluations of each of the individual polyols remain valid. That is, for each polyol, no adverse effects in humans would be anticipated at consumption levels, especially in light of the data from controlled human studies demonstrating no adverse effects following consumption of large amounts of various polyols. The foregoing has shown that there is a scientific basis for JECFA to conclude that the adrenal medullary lesions are of no relevance to humans, that the consumption of polyols is considered to be safe and free from toxic effects, and that the current JECFA recommendation of an ADI not specified for each of the polyols is appropriate.

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#### APPENDIX A: ANIMAL TOXICOLOGY STUDIES

The toxicity studies, conducted in rodents and other species, of relevance to the objectives of this report are reviewed for each of the individual polyols under consideration.

##### Sorbitol

Sorbitol has been shown, at 20% in the diet, to produce proliferative lesions in the adrenal medulla in Sprague-Dawley and Fuellinsdorf-Albino (Wistar) rats following 98-104 weeks of treatment (Hunter *et al.*, 1978a; Bär, 1984). No proliferative effects on the adrenals have been reported in a 2-year dog study (Heywood *et al.*, 1981) or in shorter-term studies in which mice were fed sorbitol at 20% of the diet for 16-24 weeks (Bär, 1983a,b). In the short-term mouse studies, 20% sorbitol in the diet was associated with an increased incidence of renal medullary and pelvic nephrocalcinosis and increased urinary excretion of calcium, magnesium, and citrate (Bär, 1983a,b). These effects were not observed in the 2-year dog study (Heywood *et al.*, 1981).

In the long-term rat study, both male and female Sprague-Dawley rats fed 20% dietary sorbitol for 2 years showed significantly increased incidence of adrenal medullary hyperplasia but no significant increase in the incidence of pheochromocytoma (Hunter *et al.*, 1978a). The incidence of adrenal medullary hyperpla-

sia was 5/60 in control males, 15/62 in sorbitol-treated males, 1/62 in control females, and 8/64 in sorbitol-treated females. A summary of the incidence of lesions of the adrenal medulla reported in control and sorbitol-treated rats in the Hunter *et al.* (1978a) study is presented in Table A1.

In addition to the reported effects on the adrenal medulla, sorbitol-treated rats were also found to have cecal enlargement.

A recent study was conducted by Hoffman-LaRoche (Bär, 1984). In this study, groups of 100 male Fuellinsdorf-Albino (Wistar) rats were fed sorbitol at a dietary concentration of 20% as part of a cereal-based pelleted diet. Controls were fed native rice starch. Treatment

TABLE A1  
Incidence of Adrenal Medullary Lesions in  
Sprague-Dawley Rats Fed Sorbitol for 2 Years

Group	Sex	No. of adrenals examined	No. with hyperplasia (%)	No. with pheochromocytoma (%)
Control	Male	60	5 (8)	3 (5)
	Female	62	1 (1.5)	1 (1.5)
20% Sorbitol	Male	62	15 (24)*	3 (5)
	Female	64	8 (12.5)*	3 (4.5)

\* Statistically significant difference from the controls of the same sex ( $P < 0.05$ ).

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**TABLE A2**  
**Incidence of Adrenal Medullary Lesions in Fuellinsdorf-Albino Rats Fed Sorbitol for 98 Weeks**

Group	No. of rats examined	No. of rats with pheochromocytoma (%)	No. with hyperplasia (severity)				Total numbers with hyper-/neoplasia (%)
			Slight	Moderate	Marked	Total (%)	
Rice starch controls	37	14 (38)	5	12	10	27 (73)	29 (78)
20% Sorbitol	34	30 (88)*	1	4	23	28 (82)	34 (100)*

\* Statistically significant difference from the controls of the same sex ( $P < 0.05$ ).

was continued for 98 weeks. Interim sacrifices were performed at Weeks 70 and 93. Bär (1984) reported a very high incidence (78%) of hyper-/neoplasia of the adrenal medulla in the rice starch-fed controls. The sorbitol-treated rats were reported to have a 100% incidence of adrenal medullary hyper-/neoplasia. Marked enlargement of the adrenal glands, increased total nor-epinephrine and dopamine content of the medullary tissue, and slight but significant increase in serum calcium levels were reported in the sorbitol-treated rats sacrificed at Weeks 70 and 93 (Bär, 1984). The proliferative lesions in the adrenal medulla were reported to display reduced chromaffinity in comparison to the surrounding normal adrenal medullary tissue. No increase in plasma catecholamine levels was observed in the sorbitol-treated rats despite the increased catecholamine content of the adrenal tissue. In contrast to the results of Hunter *et al.* (1978a), Bär (1984) reported that pheochromocytomas of the adrenal medulla of sorbitol-treated rats were also increased when compared to the concurrent controls. The incidence of hyperplasia was similar between the sorbitol-treated and control rats; however, the severity of the hyperplastic lesions was much more marked in the sorbitol-treated rats. Detailed incidence data for the hyperplastic and neoplastic lesions of the adrenal medulla reported by Bär (1984) are presented in Table A2.

In a long-term rat study on sorbitol, Gongwer and Hubben (1969) did not report any neoplastic responses in any tissues of groups of 24 male and 24 female Sprague-Dawley rats administered sorbitol at concentrations of 0, 1, 5, or 10% in a Purina Lab Chow basal diet. Mortality and food consumption were not significantly altered by treatment with sorbitol; however, male rats experienced reduced body weight gain after 1 year of treatment at the 10% level, likely due to the reduced caloric value of the sorbitol-containing diet.

### Mannitol

Long-term toxicity/carcinogenicity studies on mannitol have been conducted in the F344, Wistar, and Sprague-Dawley rat strains as well as in one mouse strain (B6C3F<sub>1</sub>) (Gongwer *et al.*, 1978; Saatman *et al.*, 1978; NTP, 1982). In the F344 rat, 10% dietary manni-

tol slightly increased the incidence of adrenal medullary proliferative lesions, an effect that was not observed in Wistar or Sprague-Dawley rats or in the B6C3F<sub>1</sub> mouse.

The long-term toxicity of mannitol in rats was first evaluated by Saatman *et al.* (1978). Groups of 40 Wistar-derived rats of each sex were administered diets containing mannitol at concentrations of 0, 1, 5, or 10% for 94 weeks. At the two higher dose levels, body weights were reduced by 5–7% in males and to a lesser extent in females. This effect is not unexpected and therefore not an adverse effect per se, given the lower caloric value of the mannitol-containing diets. A significant dose-related increase in the urinary excretion of calcium and magnesium was also observed. Histological examination of the rats revealed no significant alterations in the adrenals.

In another study, mannitol was fed in a Purina Lab Chow diet at concentrations of 0, 1, 5, or 10% to groups of 100 female F344, Sprague-Dawley, and Wistar rats for 30, 27, and 30 months, respectively (Gongwer *et al.*, 1978). In the treated rats, there was a slight trend to reduced body weight gain and a tendency for increased urinary concentrations of calcium. Analysis of the incidence of adrenal lesions indicated that focal medullary hyperplasia was significantly increased in the high-dose F344 rats (9/96 in the treated rats and 2/99 in the controls). The incidence of unilateral pheochromocytoma was also higher in F344 rats given 10% dietary mannitol, but this effect was not statistically significant (26/96 in treated rats vs 15/99 in controls). The 1 and 5% mannitol groups showed a lower incidence of pheochromocytoma than the controls. No effects of treatment on the adrenal medulla were evident in the Sprague-Dawley or Wistar rat.

As part of the National Toxicology Program testing program (NTP, 1982), D-mannitol was tested for carcinogenicity in F344 rats and B6C3F<sub>1</sub> mice. In this study, conducted under the standard NTP protocol, groups of 50 male and 50 female rats and mice were fed D-mannitol at 0, 2.5, or 5.0% of a standard NTP laboratory chow diet for 103 weeks. No carcinogenic effects were observed in either rats or mice. In general, there were no effects of treatment on body weight gain or on mortality.

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**TABLE A3**  
**Incidence of Adrenal Medullary Lesions in**  
**Sprague-Dawley Rats Fed Xylitol for 2 Years**

Group	Sex	No. of rats examined	No. with tumors	No. with hyperplasia
Controls (0% xylitol)	Male	60	3	5
	Female	62	1	1
20% sucrose	Male	57	1	4
	Female	62	2	2
2% Xylitol	Male	60	1	6
	Female	63	1	1
5% Xylitol	Male	59	1	8
	Female	61	2	8*
10% Xylitol	Male	62	2	12*
	Female	64	1	7*
20% Xylitol	Male	61	8*	16*
	Female	65	4	11*
20% Sorbitol	Male	62	3	15*
	Female	64	3	8*

\* Statistically significant difference from the controls of the same sex ( $P < 0.05$ ).

### Xylitol

Xylitol, at high concentrations in the diet (i.e., 20%), has been demonstrated to be associated with an increase in the incidence of proliferative lesions of the adrenal medulla in Sprague-Dawley and Wistar rats (Hunter *et al.*, 1978a; Bär, 1984, 1986).

No effects on the adrenal medulla have been observed in mice fed xylitol at up to 20% in the diet for 2 years (Hunter *et al.*, 1978b). Xylitol fed to dogs at 0, 2, 5, 10, or 20% of a rice starch diet for 2 years also failed to elicit any proliferative responses in adrenal medullary tissue (Heywood *et al.*, 1977).

In the first long-term feeding study in rats, groups of 75 male and 75 female Sprague-Dawley rats were fed xylitol in the diet at concentrations of 0, 2, 5, 10, or 20% for a 2-year period (Hunter *et al.*, 1978a). As a result of treatment, a significant increase in the incidence of hyperplasia of the adrenal medulla was observed in the 10 and 20% dose group males (12/62 and 16/61, respectively vs 5/60 in the controls), and 5, 10, and 20% dose group females (8/61, 7/64, and 11/65, respectively vs 1/62 in the controls). In the male rats administered 20% xylitol, the incidence of pheochromocytomas (8/61) was also significantly increased compared to controls (3/60). Body weight gain was reduced, compared to controls, in the rats fed xylitol at greater than 2% in the diet. As discussed with mannitol and sorbitol, slight reductions in body weights are likely the result of the reduced caloric values of the polyol-containing diets, and not an adverse effect per se. A summary of the proliferative lesions of the adrenal medulla reported in xylitol-fed and control rats of the Hunter *et al.* (1978a) study is given in Table A3.

In a second study, Bär (1984) fed a group of 100 Fuellinsdorf-Albino (Wistar) rats a cereal-based diet containing 20% xylitol or 20% native rice starch (control group) for a 98-week period. In the xylitol-treated animals, multiple proliferative lesions of the adrenal medulla were observed (incidence of 100%), although the control group also showed a high incidence of proliferative lesions (78%). In addition, chemical analysis of some of the adrenal glands indicated that xylitol treatment resulted in an increase in total adrenal norepinephrine and dopamine content. Plasma calcium levels were also slightly, but significantly, increased in the xylitol-treated rats. The results of the Bär (1984) bioassay on xylitol were similar to the results of the previously discussed Bär (1984) bioassay on sorbitol.

In a third long-term study, the effect of dietary calcium on the occurrence of adrenal lesions with xylitol treatment was investigated (Bär, 1986). Groups of 75 male Fuellinsdorf-Albino (Wistar) rats were fed diets containing 20% xylitol and 0.4, 0.2, or 0.05% calcium as the carbonate salt. Controls received 0% xylitol and 0.4% calcium in the diet. Treatment was continued for a period of 63 weeks. At histopathological examination, the incidence of tumors of the adrenal medulla was significantly increased in the xylitol-treated animals given 0.2 and 0.4% dietary calcium. Incidences are presented in Table A4.

In addition, by Week 13/14 urinary calcium excretion was considerably greater in the xylitol-fed rats administered a diet containing more than 0.05% calcium. By Week 53/54, urinary excretion of calcium was greater than that of controls in all groups of xylitol-fed rats.

### Lactitol

One long-term toxicity/carcinogenicity rat assay has been conducted on lactitol using the Wistar rat strain and has shown that high dietary concentrations of lactitol (i.e., 10%) and lactose (i.e., 20%) increase the incidence of proliferative lesions of the adrenal medulla, especially in males (Sinkeldam *et al.*, 1983, 1992; Woutersen, 1987). The results of a long-term bioassay in mice, with lactitol concentrations of 2–10% of the diet, showed no proliferative effects on the adrenal medulla (Til *et al.*, 1992). In fact, beyond increased cecal weights (both full and empty) in the 5 and 10% dose groups, no pathological changes could be ascribed to lactitol treatment. Similarly, no pathological changes beyond cecal enlargement were reported in dogs fed lactitol at concentrations of up to 15% of the diet for 26 weeks (Til *et al.*, 1992).

In the Sinkeldam *et al.* (1992) chronic rat study on lactitol, groups of 50 male and 50 female SPF-Wistar rats were fed lactitol, in place of sucrose and lactose, at concentrations of 0, 2, 5, or 10% in a basal CIVO diet for a period of 30 months. As a reference control, a group of 50 male and 50 female rats received 20%

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TABLE A4  
Adrenal Medullary Lesions in Xylitol-Fed Rats Given Different Dietary Calcium Levels

Group	Treatment	No. of animals	No. with hyperplasia	No. with tumors
Control	0% Xylitol/0.4% Ca <sup>2+</sup>	63	40	6
1	20% Xylitol/0.4% Ca <sup>2+</sup>	56	31	24*
2	20% Xylitol/0.2% Ca <sup>2+</sup>	57	39	17*
3	20% Xylitol/0.05% Ca <sup>2+</sup>	63	55	7

\* Statistically significant difference from controls ( $P < 0.05$ ).

lactose in the diet at the expense of sucrose and wheat starch. All of the animals were previously exposed to their respective diets *in utero*. At histopathological examination, a statistically significant increase in the incidence of adrenal medullary hyperplasia was observed in the 10% lactitol and 20% lactose groups. A significantly increased incidence of pheochromocytoma (benign and malignant combined) was also reported in the 10% lactitol and the 20% lactose males. The incidence of malignant pheochromocytoma was not significantly different between the treated groups and controls (Sinkeldam *et al.*, 1992). Significant nonneoplastic effects reported in the Sinkeldam *et al.* (1992) study included reduced body weight gain in the high-dose lactitol and lactose-treated rats, calcification of the renal pelvis in the 10% lactitol and 20% lactose males and in the 5 and 10% lactitol-treated females, and a dose-related increase in urinary calcium excretion with 20% lactose having the greatest effect.

The results of a Japanese 52-week chronic rat bioassay on lactitol were published (Okazaki *et al.*, 1994). Twenty-five male and 25 female Sprague-Dawley rats (Slc) were administered lactitol in the diet at doses of 0, 0.4, 2, or 10 g/kg body wt/day. Following a 52-week treatment period, the rats were allowed to recover for 9 weeks. In the high-dose group, treatment-related mortality, decreased body weight gain and food intake, and dilatation of the renal tubules were reported (Okazaki *et al.*, 1994). Increased calcium excretion and increased cecal weights were reported in the 2 and 10 g/kg body wt/day dose groups. One pheochromocytoma was reported in a low-dose male. No other hyper-/neoplastic lesions of the adrenal medulla were recorded; however, the adrenal weights were increased in both sexes of the 10 g/kg body wt/day dose group. The effects reported in the study were all reversible, except for cecal enlargement in the top dose animals, following the 9-week recovery period. Okazaki *et al.* (1994) reported a no-observed-adverse-effect level of 0.4 g/kg body wt/day.

#### Maltitol

The results of a long-term/carcinogenicity study conducted on a preparation containing 87% maltitol have been recently reviewed by JECFA (1993). In this study

(Conz and Fumero, 1989; Conz and Maraschin, 1992), the preparation was administered in the diet at doses of 0, 0.5, 1.5, or 4.5 g/kg body wt/day (about 1–10% in the diet) to groups of 70 male and 70 female Sprague-Dawley [CrI:CD(SD) BR] rats. Twenty rats of each group were sacrificed at 52 weeks and the remainder of the animals were left on the carcinogenicity study for 106 weeks.

In the first 52 weeks, no significant adverse effects occurred in any of the treated groups. Cecal enlargement, an effect of no toxicological significance, occurred in the high-dose males likely due to the increased osmotic value of the cecal contents resulting from the unabsorbed maltitol. Histopathological examination at the end of the carcinogenicity study revealed treatment-related effects on the adrenal gland. These effects included increased incidence of both benign and malignant pheochromocytomas in the high-dose animals of both sexes and an increased frequency of slight to moderate adrenal medullary hyperplasia in all treated groups. The incidence of the histopathological effects of maltitol treatment on the adrenal glands of rats is presented in Table A5.

No long-term beagle dog or mouse studies on pure maltitol have been reported in the scientific literature.

#### Maltitol Syrup (Hydrogenated Glucose Syrups)

Several subchronic/chronic toxicity studies (Wada, 1972; Yamasaki *et al.*, 1973; Shimpo, 1977; Shimpo *et al.*, 1977; Dupas and Leroy, 1984) have been conducted on maltitol syrups, formerly known as hydrogenated glucose syrups, which contain 50–98% maltitol as well as sorbitol, and other polysaccharide-derived sugar alcohols. Furthermore, maltitol syrups belong to a family of closely related polysaccharide-derived polyols commonly referred to as HSHs. HSHs that do not meet the specifications for maltitol syrups (i.e., do not have a maltitol content of 50–98%), such as sorbitol syrups, are expected to have a toxicological profile similar to that described for maltitol syrups because HSH products contain the same constituents as maltitol syrups, namely, polysaccharide components, and varying amounts of sorbitol and maltitol, only at different concentrations.

Shimpo and colleagues (Shimpo, 1977; Shimpo *et al.*,

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**TABLE A5**  
**Histopathological Changes in the Adrenal Gland of Rats Administered Maltitol in the Diet**

Lesion type	Sex and dose level (g/kg body weight/day) <sup>a</sup>							
	Males				Females			
	0	0.5	1.5	4.5	0	0.5	1.5	4.5
Pheochromocytoma								
Benign	8	4	10	20*	2	2	4	10*
Malignant	6	12*	4	10*	2	2	2	4*
Combined	14	16	14	30*	4	4	6	14*
Medullary hyperplasia	24	32	38	32	14	22	24	34*

<sup>a</sup> Fifty adrenals per group were examined, except high-dose males, in which 49 were examined.

\* Statistically significant from controls ( $P < 0.05$ ).

1977) fed a maltitol syrup (Multi-Towa), containing more than 75% maltitol, to groups of 52 male and 52 female Wistar rats at dose levels of 0, 3, or 10% of the diet for a period of 78 weeks. Toward the end of the study, a trend for reduced growth was seen in the 3% males and in both sexes of the 10% dose group. This was likely due to the reduced caloric value of the maltitol syrup-containing diet. No treatment-related histopathological changes in the adrenal medulla were reported (Shimpo, 1977; Shimpo *et al.*, 1977). In the adrenal cortex, one adenoma was reported in the high-dose males (1/33), with none in the controls (0/28) and 3/6, 5/29, and 6/33 female rats were found to have an adrenocortical adenoma in the control, 3%, and 10% dose groups, respectively. The slight increase of adrenocortical tumors was also reported in JECFA (1987). The incidence of various age-related nonneoplastic and neoplastic lesions was similar in the control and treated rats.

No adrenal medullary lesions were reported in a 24-month toxicity study in which groups of 50 male and 50 female Sprague-Dawley rats were administered a maltitol syrup (Lycasin 80/55) containing 52.5% maltitol, 7.5% sorbitol, and 40% higher-order hydrogenated saccharides at a dose of 18,000 ppm in the drinking water (Dupas and Leroy, 1984). The drinking water dose resulted in exposures of 13.9 and 21.5 g/kg body wt/day for males and females, respectively. As a result of treatment, mortality and food consumption were decreased and water consumption increased, whereas no effect on body weight gain occurred except for an adaptation period at the start of the study. As reported with other poorly absorbed carbohydrates, dosing with the maltitol syrup resulted in cecal enlargement. Cecal enlargement, in the context of polyol consumption, is of no toxicological significance. No significant differences were reported between the treated and control groups with respect to the occurrence of neoplastic and non-neoplastic lesions. A 5–10% increase in serum calcium levels was reported in the treated rats, with this effect

reaching statistical significance in females sacrificed after 20.5 months on study and in both sexes following 24 months of treatment.

No adrenal lesions were reported in rats following 31-week administration of maltitol syrup at concentrations of up to 20% in the diet or of maltitol at up to 30% in the diet (Wada, 1972). Similarly, no adrenal medullary lesions were reported to occur in a 13-month study in which Wistar rats were administered diets containing up to 10% maltitol syrup (Malbit) (Yamasaki *et al.*, 1973).

#### Isomalt

Isomalt has been subject to long-term toxicity/carcinogenicity studies with rats (Smits-Van Prooije *et al.*, 1990), mice (Smits-Van Prooije *et al.*, 1990), and dogs (Hoffmann *et al.*, 1981). Isomalt fed to Wistar rats at dietary concentrations of 0, 2.5, 5, or 10% for 128–130 weeks was reported not to be associated with an increased incidence of adrenal medullary proliferative lesions (Smits-Van Prooije *et al.*, 1990). The rats in this study also had been exposed to their diets *in utero*. No indications of proliferative effects on the adrenal medulla were reported in chronic studies with mice or dogs fed isomalt at concentrations of up to 10% in the diet.

In the chronic rat study, a slight reduction of body weight gain was seen in the 5% isomalt females and the 10% isomalt males (Smits-Van Prooije *et al.*, 1990). Body weight reduction was to be expected, and therefore not an adverse effect, given the lower caloric value of the isomalt diets and the lack of increased rates of food consumption to counter the reduced caloric value of the diet. Enlargement of the cecum in the 10% isomalt male rats was also reported. As discussed with other polyols, cecal enlargement is not a toxicologically significant effect because it is likely the result of increased osmotic pressure within the cecum resulting from the unabsorbed and fermented portions of isomalt

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**TABLE A6**  
**Histopathological Changes in the Adrenal Gland of Rats Administered Basal Diet, Lactose, Lactose and Ammonium Chloride, and Lactose and Potassium Bicarbonate**

Lesion type	Sex and treatment group <sup>a</sup>							
	Males				Females			
	Basal diet	20% Lactose			Basal diet	20% Lactose		
		Alone	+ 1% NH <sub>4</sub> Cl	+ 2% KHCO <sub>3</sub>		Alone	+ 1% NH <sub>4</sub> Cl	+ 2% KHCO <sub>3</sub>
Pheochromocytoma								
Single benign	13	13 <sup>b</sup>	5 <sup>c</sup>	10	2	0	0	1
Multiple benign	1	1 <sup>b</sup>	4	9 <sup>c</sup>	0	0	0	2
Malignant	0	1	3	2	0	0	1	1
Total	14	15	11 <sup>b</sup>	21 <sup>c</sup>	2	0	1	4
Basophilic foci (focal hyperplasia)	9	16 <sup>b</sup>	3 <sup>c</sup>	8	2	1	1	5
Pheochromocytoma and basophilic foci combined	19 <sup>b</sup>	27 <sup>c</sup>	14 <sup>b</sup>	25 <sup>c</sup>	4	1 <sup>b</sup>	2	8 <sup>c</sup>

<sup>a</sup> Fifty adrenals per group were examined, except basal diet males, in which 49 were examined.

<sup>b,c</sup> Values in a row for each sex column with different letters differ significantly from one another according to the Fischer exact probability test.

remaining in the colon. Overall, the results of the chronic rat study indicate that no significant toxicological effects occur following long-term exposure to isomalt at up to 10% in the diet.

Similarly, in mice fed isomalt at dietary concentrations of 0–10% for nearly 2 years, no significant adverse effects occurred in any of the treatment groups (Smits-Van Prooije *et al.*, 1990). Slight body weight gain reduction, likely resulting from the reduced caloric value of the isomalt-containing diets, was reported in the 5 and 10% isomalt females. Increases in the absolute and relative weight of the cecum in high-dose animals of both sexes were also reported.

### Lactose

Lactose has been shown to produce increased incidences of hyperplastic and neoplastic lesions of the adrenal medulla in rats (Sinkeldam *et al.*, 1983, 1992; Woutersen, 1987) but not in mice (Til *et al.*, 1986).

In the rat study, in which 50 male and 50 female SPF-Wistar rats were fed lactose at the expense of sucrose and wheat starch, at a concentration of 20% in the diet for a period of 30 months, a statistically significant increase in the incidence of adrenal medullary hyperplasia was observed in both sexes, with an increased incidence of combined benign and malignant pheochromocytoma reported in males. The lactose-treated rats were also reported to show reduced body weight gain, calcification of the renal pelvis, and increased urinary calcium excretion.

An 89-week mouse study showed no deleterious ef-

fects on the adrenal medulla as a result of feeding lactose at a dietary concentration of 55% (Til *et al.*, 1986). Lactose treatment of albino SPF mice resulted in slightly lower body weights, increased urinary calcium levels, renal tubular calcinosis, and concrements in the pelvic space. Cecal enlargement, commonly observed in laboratory animals following high dietary intake of poorly absorbed carbohydrates (an effect resulting from the increased osmotic value of the cecal contents), was also reported to occur in the lactose treated mice.

Another study examining the modulating effects of a diet load of acid or base on the toxicity of lactose has been published (de Groot *et al.*, 1995). de Groot *et al.* (1995) fed a basal diet to one group of 50 male and 50 female rats (Wistar derived) and a basal diet containing 20% lactose to three groups of 50 rats of each sex. Two of these three groups also received an acidifying agent (1% ammonium chloride) or an alkalinizing agent (2% potassium bicarbonate). All groups were treated for up to 30 months. Lactose feeding, regardless of the presence of acidifying or alkalinizing agents, was associated with reduced mortality compared to controls, reduced fecal pH, higher water and food intakes, lower body weights, and increased cecal weights. Lactose consumption was also associated with decreased severity of nephrosis, increased mineralization, and hyperplasia of the epithelium of the renal pelvis. Urinary calcium levels were also increased by lactose treatment; however, cotreatment with ammonium chloride enhanced this effect, whereas cotreatment with potas-

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sium bicarbonate reduced this effect. With respect to the adrenal medulla, lactose treatment alone did not result in an increased incidence of pheochromocytoma compared to treatment with the basal diet. The authors reported that the incidence of basophilic foci (focal hyperplasia) in the adrenal medulla, however, was significantly increased in the lactose-treated animals. de Groot *et al.* (1995) also reported that lactose and ammonium chloride treatment resulted in a decreased incidence of basophilic foci and of multiple pheochromocytoma. Treatment with 20% dietary lactose and 2% potassium bicarbonate was reported by the authors to result in a significant increase in the incidence of multiple pheochromocytoma. The incidence of lesions of the adrenal medulla in the various treatment groups, reported by de Groot *et al.* (1995), is presented in Table A6.

On the basis of the available data, it is clear that acidification of the diet may result in decreased incidence of hyperplastic and neoplastic lesions of the adrenal medulla in rats administered lactose. However, due to the lack of clear relationships among the various dose groups and the basal diet controls, and given the known variability in the spontaneous incidence of adrenal medullary tumors in rats, it is difficult to determine the significance of the results of the de Groot *et al.* (1995) experiment in terms of the potential differential effects of certain polyols on the adrenal medulla of rats and humans. Moreover, although it has been suggested that fermentation of polyols in the lower gut could result in an increased acid load to the body (de Groot *et al.*, 1995), with subsequent hypercalciuria, it is not possible to determine if the direct acid/base loading via the diet as used in the de Groot *et al.* (1995) study appropriately mimics the potential acidification that could hypothetically occur from the fermentation of dietary polyols to short-chain volatile fatty acids.

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## APPENDIX B: HUMAN STUDIES

Several human studies have been conducted on polyols that have assessed the safety, cariogenicity, caloric value, and metabolism of polyols in man.

### Xylitol

One 2-year clinical study on the cariogenicity of xylitol, in which three groups of volunteers remained on strict diets containing fructose ( $n = 35$ , average consumption of 2.1 kg/month), sucrose ( $n = 38$ , average consumption of 2.2 kg/month), and xylitol ( $n = 52$ , average consumption of 1.5 kg/month), reported that measures of urinary sodium, potassium, calcium, magnesium, phosphate, ascorbate, bilirubin, amylase, SAP, amino acids, and immunoglobulins were unaffected by consumption of xylitol (Scheinin and Mäkinen, 1975). There were also no significant differences in body weight changes between the different dietary groups. In all groups, pregnancies that occurred over the 2-year period proceeded normally (Scheinin and Mäkinen, 1975). Further follow-ups of members of the cohort also failed to show any ad-

verse effect of xylitol on parameters indicative of hepatobiliary toxicity or metabolic intolerance (Mäkinen and Virtanen, 1978).

An examination of the effects of a high xylitol dietary load in some of the xylitol-consuming subjects of the Scheinin and Mäkinen (1975) and Mäkinen and Virtanen (1978) studies was performed by Mäkinen *et al.* (1981). In this study, nine subjects who had regularly consumed substantial amounts of xylitol for 4.3-5.3 years without adverse effect participated in a feeding study involving daily consumption of between 70 g (females) and 100 g (males) of xylitol and sucrose for periods of 14 and 7 days, respectively. During each period of the study, blood and urine samples were collected for measurements of a number of biochemical, hematological, and microscopic determinations of various parameters to identify any effect of xylitol consumption on the metabolism of lipids, carbohydrates, uric acid, oxalic acid, urinary catecholamines, or electrolytes. The 7-day sucrose and 14-day xylitol treatment periods had no significant impact on any of the hematological or biochemical parameters measured. There was no evidence of any xylitol-related effects on oxalic acid excretion, urine pH, catecholamine levels, or electrolyte bal-

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ance, including calcium (Mäkinen *et al.*, 1981). As a result, Mäkinen *et al.* (1981) concluded that there was no evidence to suggest xylitol consumption has an adverse effect on liver function or produces symptoms indicative of hyper-neoplasia of the adrenal medulla.

In another cariogenicity study, a cohort of 800 7- to 13-year-old Hungarian school children consuming xylitol as part of a caries prevention programme was followed for up to 3 years (Bär, 1983, 1984). In this study, measurements of a number of urinary and plasma parameters were obtained prior to the start of treatment and after 1, 2, and 3 years of consumption of products sweetened with xylitol. The results of these analyses demonstrate that long-term xylitol consumption had no effect on urinary parameters related to liver, kidney, or metabolic dysfunction. Throughout the study, there were also no complaints of gastrointestinal side effects that could be attributed to xylitol consumption.

In a third cariogenicity study, two groups of Polyneesian school children were followed for 32 months, with one group consuming xylitol-sweetened snacks (up to 20 g of xylitol per day) and another following their normal snacking habits (controls) (Bär and Lamm, 1986). Plasma samples obtained from 448 of the xylitol-consuming children and 351 of the controls showed no differences with respect to AP, GPT, GOT,  $\gamma$ -GT, GLDH, cholinesterase, uric acid, and bilirubin concentrations. As a result, there appeared to be no significant change in hepatobiliary function as a result of long-term consumption of xylitol. Similarly, other than a transient increase in glycolate concentration, urinalysis parameters were not significantly different between the two groups of children; thus, xylitol consumption did not affect risk factors known to be associated with urolithiasis.

The effects of xylitol consumption on urinary calcium excretion were examined in a double-blind study in which 12 human volunteers received dietary supplements of sucrose or xylitol at a dose of 1 g/kg body wt for a 1-week period each (crossover design) (Bär, 1985). The 24-hr calcium excretion did not differ between the two treatment periods.

Studies of shorter duration have shown xylitol to be well tolerated, at least to a dose of 30 g/day (Amador and Eisenstein, 1971; Asano *et al.*, 1972; Mertz *et al.*, 1972; Huttunen *et al.*, 1975; Förster *et al.*, 1977, 1981; Akerblom *et al.*, 1981; Hassinger *et al.*, 1981; Wang *et al.*, 1981; Culbert *et al.*, 1986). These studies, several of which were performed in diabetic subjects, also demonstrated that consumption of large amounts of xylitol is not associated with metabolic intolerance or with any significant alterations of energy metabolism or mineral homeostasis.

#### Lactitol

Lactitol has been reported to be well tolerated by healthy and diabetic subjects consuming lactitol at

rates of up to 24 g/day (Doorenbos, 1977; Zaal and Ottenhof, 1977). No effects on blood glucose or insulin levels were reported in diabetic subjects (Doorenbos, 1977). Loading tests conducted with equal proportions of sucrose, lactose, lactitol, and lactitol and sucrose in eight healthy volunteers demonstrated that elevations in blood glucose were attenuated in subjects consuming lactitol compared with sucrose and lactose (Zaal and Ottenhof, 1977). Several of the subjects who consumed 50 g of lactitol complained of gastrointestinal discomfort, including diarrhea (Zaal and Ottenhof, 1977). As indicated previously, the occurrence of diarrhea is not an adverse effect per se, but simply a transient osmotic effect that occurs during the adaptation of the gut microflora.

Two studies have examined the effects of lactitol consumption on calcium metabolism. The effect of lactitol on calcium metabolism was investigated in a crossover study conducted by Egger *et al.* (1989). In this study, 12 volunteers received either 20 or 40 g of lactitol daily for a 1-month period. A control group received no lactitol supplement. Urinalysis tests showed that lactitol ingestion had no effect on urinary excretion of calcium or inorganic phosphate. Also, plasma levels of calcium, phosphate, SAP, PTH, and osteocalcin were unaffected by the treatment.

In another study, Griessen *et al.* (1989), using a double isotope technique, evaluated the effect of glucose, galactose, and lactitol on intestinal calcium absorption in healthy male volunteers. Although the addition of 25 g of glucose or galactose was reported to increase the fractional calcium absorption by 30%, lactitol, given at a dose of 15 g, reduced the intestinal absorption of calcium by 15%. Taken together, these studies indicate that lactitol has no significant effect on calcium homeostasis in humans.

#### Maltitol

There are no available longer-term human studies on maltitol per se because most of the longer-term human studies were conducted using maltitol syrups. Short-term metabolic and tolerance tests on maltitol (Mimura *et al.*, 1972; Kamoi *et al.*, 1975; Atsuji *et al.*, 1982) have been conducted in both healthy and diabetic subjects. These studies, like similar studies on xylitol and lactitol, show maltitol to be well tolerated and to have little effect on blood glucose levels or on fat, lipid, or carbohydrate metabolism. Based on these data and data on maltitol syrups (reviewed in the following section), it is apparent that maltitol produces no toxicologically significant effects in humans even when consumed in large amounts.

#### Maltitol Syrup (Hydrogenated Glucose Syrups)

Two longer-term studies on maltitol syrup (Lycasin 80/55), one of 30 days duration with 5 healthy male

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subjects (Itoya *et al.*, 1974) and the other of 4 months duration with 107 male (11 diabetics) and 20 female (two diabetics) subjects (Tacquet and Devulder, 1978), have demonstrated that maltitol syrup consumption at levels of up to 90 g per day has no effect on various biochemical parameters, including serum concentrations of protein, cholesterol, bilirubin, uric acid, urea nitrogen, SGOT, SGPT, LDH, sodium, potassium, and calcium (Itoya *et al.*, 1974; Tacquet and Devulder, 1978). The human data on maltitol syrup indicate that consumption of up to 90 g per day produces no significant adverse effects on hepatobiliary function, metabolism of other nutrients, and mineral homeostasis.

Short-term metabolic and tolerance tests on maltitol syrup (formerly known as hydrogenated glucose syrup) (Abraham *et al.*, 1981; Kearsley *et al.*, 1982; Secchi *et al.*, 1982) have been conducted in healthy subjects. These studies, like similar studies on other polyols, including xylitol, lactitol, and maltitol, indicate maltitol syrup to be well tolerated and to have little effect on blood glucose levels or on fat, lipid, or carbohydrate metabolism.

Also, several other studies [reviewed in Modderman (1993) and in FASEB (1994)] on the metabolic and short-term effects of hydrogenated starch hydrolysate products containing less than 50% maltitol (i.e., not meeting the JECFA specifications for "maltitol syrups"), which are otherwise qualitatively similar to maltitol syrups, have also demonstrated that there are no adverse effects in humans of these polysaccharide-derived polyol mixtures, even at relatively high rates of consumption.

### Isomalt

Several medium-term studies have been published that document the tolerance of humans, both healthy and diabetic, to long-term consumption of isomalt at rates of up to 48 g/day. The effects of a 12-week administration of isomalt on metabolic control in type II diabetics were investigated in 24 subjects following administration of 24 g of isomalt daily (Pometta *et al.*, 1985). No differences were found between the isomalt and the control group for blood glucose, hemoglobin HbA<sub>1c</sub>, and serum lipids (i.e., cholesterol, triglycerides, and HDL cholesterol). Renal and liver function tests and blood count were in the normal range throughout the study period and no differences were observed between the isomalt and the control group (Pometta *et al.*, 1985). Pometta *et al.* (1985) concluded that isomalt was well tolerated and did not result in any metabolic disturbances following consumption at rates of 24 g/day. This conclusion echoed the results of shorter-term and acute studies showing that isomalt consumption at up to 50 g/day had no effects on urinalysis and had hematological parameters indicative of disturbances of carbohydrate metabolism (Bachmann *et al.*, 1984; Thiébaud *et al.*, 1984).

In another 12-week study (Spengler *et al.*, 1987), isomalt was given in increasing doses in the form of various foods to a group of 30 healthy volunteers. Another group of 30 volunteers received corresponding doses of sucrose in the same foods given to the isomalt group. Daily doses increased from 12 to 48 g for both sucrose and isomalt. The results of the Spengler *et al.* (1987) study confirmed the results of the Pometta *et al.* (1985) study, thus indicating that isomalt is well tolerated over long periods, even at high levels of consumption.

Other short-term metabolic and tolerance tests on isomalt (Keup and Putter, 1974; Putter and Spengler, 1975; Siebert *et al.*, 1975; Spengler, 1978; Spengler and Schmitz, 1979), previously reviewed by JECFA (1981, 1987), indicate isomalt to be well tolerated and to have little to no effect on blood glucose levels or on fat, lipid, or carbohydrate metabolism.

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## APPENDIX C: METABOLISM STUDIES

As a result of structural and metabolic similarities, the various polyols can be divided into the monosaccharide-derived polyols, the disaccharide-derived polyols, and those that are derived from mixtures of mono-, di-, and polysaccharides. Several reviews concerning the absorption and metabolism of polyols and lactose are available (FASEB, 1986, 1994). A brief overview of the absorption, distribution, and metabolism of the individual polyols under consideration is presented here followed by a summary of the data in terms of assessing the relevance to humans of the adrenal medullary tumors observed in rats following long-term high-dose dietary exposure to certain polyols.

*Sorbitol*

Following oral administration of [ $^{14}\text{C}$ ]sorbitol (35 g) to diabetics or volunteers, 75% of the label was rapidly excreted as  $^{14}\text{CO}_2$  and 3% in the urine, whereas no radiolabel was found in the feces (Adcock and Gray, 1957) suggesting substantial absorption and metabolism. Beaugerie *et al.* (1990) indicated that 79% of an oral dose of sorbitol (30 g) was absorbed following recovery by ileal aspiration. No sorbitol was found in the feces. Langkilde *et al.* (1994) recovered 27% of an oral dose of sorbitol (15 g) from the distal ileum of ileostomy patients indicating that 73% of the dose was absorbed. Dehmel *et al.* (1969) administered 5 g sorbitol intraduodenally to volunteers with a temporarily blocked distal duodenum and found the dose disappeared at 12–14% the rate of glucose disappearance. Similar results were noted with rats administered 1.3 g of sorbitol. Ruttloff and Ketz (1961) found that the absorption of sorbitol (45 mg) directly injected into the small intestine of female Wistar rats was 36% that of glucose. Figdor *et al.* (1987) recovered 2.6% of the radiolabel in urine, 14% in the feces, and 48% in breath  $\text{CO}_2$  following oral administration of [ $\text{U-}^{14}\text{C}$ ]sorbitol to fasted, unadapted male Sprague-Dawley rats. Following intravenous administration, 14% of the label was recovered in the urine, 6% in the feces, and 41% in breath  $\text{CO}_2$ . The production of breath  $^{14}\text{CO}_2$  was rapid following both routes of administration indicating that most of the radiolabeled  $\text{CO}_2$  was derived from absorption and subsequent host metabolism rather than from fermentation processes. The previous results indicate that most of the orally administered sorbitol is absorbed from the small intestine.

Fermentation of sorbitol in the lower gut has been confirmed with the measurement of breath hydrogen following oral doses ranging from 5 to 30 g (Hyams, 1983; Beaugerie *et al.*, 1991; Wursch *et al.*, 1989; Lee *et al.*, 1994).

Following absorption, sorbitol is primarily metabolized in the liver, where it undergoes a iditol dehydroge-

nase catalyzed dehydrogenation reaction to form fructose (Allison, 1979a). Fructose is phosphorylated to fructose-1-phosphate that enters the normal glycolysis pathway.

*Mannitol*

A review of the older literature concerning the absorption and metabolism of mannitol is presented by Allison (1979b). Mannitol is absorbed from the upper portion of the small intestine by passive diffusion (Ruttloff and Ketz, 1961; Fordtran *et al.*, 1965; Launiala, 1968). The absorption of mannitol from the small intestine is relatively slow compared to that of glucose (active transport); the absorption may be approximately 9–45% that of glucose (Ruttloff and Ketz, 1961; Launiala, 1968). Saunders and Wiggins (1981) recovered 74% of the mannitol at the end of the small intestine following oral administration of 10 g of mannitol to fasted ileostomy patients suggesting that 26% was absorbed. Nasrallah and Iber (1969) recovered 18% of an oral dose of [ $\text{U-}^{14}\text{C}$ ]mannitol (28–100 g) in the urine and 31.5% in the feces following oral administration to patients. Elia *et al.* (1987) recovered 20% of an orally administered dose of uniformly labeled [ $^{14}\text{C}$ ]mannitol in the urine of several normal adults and ileostomy patients. Wick *et al.* (1954) recovered 8–13% of the orally administered dose of [ $^{14}\text{C}$ ]mannitol in the urine of albino rats. These studies indicate that only a small portion of the administered dose of mannitol is absorbed from the small intestine and that the largest proportion enters the colon.

Following absorption, mannitol proceeds to the liver where it is freely absorbed by the hepatocytes (Cahill *et al.*, 1958). Other cells like the erythrocyte appear to be impermeable to mannitol (LeFevre and Davies, 1951) and suggest that the liver is the primary site of metabolism. Indeed, intravenous administration of [ $\text{U-}^{14}\text{C}$ ]mannitol that would largely bypass the liver prior to rapid renal excretion (Ginn, 1974) results in small amounts of  $^{14}\text{CO}_2$  formation (1–2%) (Wick *et al.*, 1954; Nasrallah and Iber, 1969). Although several mannitol dehydrogenating enzymes have been detected, the enzyme systems that act on mannitol have not been studied. Isolated sorbitol dehydrogenase shows limited activity toward mannitol (dehydrogenated to fructose) suggesting that mannitol is not metabolized extensively. This has been confirmed in recovery studies of  $^{14}\text{CO}_2$  from orally administered uniformly labeled mannitol in rats (Wick *et al.*, 1954) and humans (Nasrallah and Iber, 1969; Elia *et al.*, 1987). Only small amounts of  $^{14}\text{CO}_2$  were recovered during the first 6 hr following ingestion suggesting that little of the absorbed mannitol is metabolized or utilized. Larger amounts of  $\text{CO}_2$  were detected after 6 hr suggesting that the mannitol entering the colon is fermented by the microorganisms found in the lower gut (Wick *et al.*, 1954; Nasrallah and Iber, 1969).

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### Xylitol

Schmidt *et al.* (1964) estimated that 20–25% of an oral dose (131–431 mg/kg body wt) of [ $^{14}\text{C}$ ]xylitol was absorbed from the small intestine of Sprague-Dawley rats. These results were confirmed by the studies of Dehmel *et al.* (1969) at significantly larger oral doses (1.3 g/rat). Muller-Hess *et al.* (1975) estimated that 25% of 30- to 50-g doses were absorbed based on indirect calorimetry studies in human volunteers. Similarly, Dehmel *et al.* (1969) indicated that 12–14% of an intraduodenal injected dose (5 g) disappeared within 1 hr from the small intestine of human volunteers with a distally blocked duodenum. In contrast, Asano *et al.* (1973) found that 72–95% of a 5- to 15-g oral dose had disappeared from ileal aspirates. Higher dosages of 15–30 g yielded apparent absorptions of 49–66%. Only small quantities of xylitol have been recovered in the feces of rats fed xylitol (Schmidt *et al.*, 1964) suggesting that the unabsorbed portion is fermented by bacteria in the colon.

Following absorption, xylitol is primarily metabolized in the liver (Bassler, 1969) by L-iditol dehydrogenase to form xylulose, which in turn is phosphorylated to xylulose-5-phosphate and enters the pentose phosphate pathway to eventually enter glycolysis or gluconeogenesis pathways as triose phosphate (Touster, 1975; Dwivedi, 1977; FASEB, 1978, 1986; Dills, 1989).

### Lactitol

Several *in vitro* and *in vivo* studies indicate that lactitol is not absorbed from the small intestine. These studies range from *in vitro* studies that indicate that lactitol is resistant to the digestive enzymes of the small intestine (Nilsson and Jagerstad, 1987; Harju, 1988a,b) that are required prior to the absorption of disaccharides to *in vivo* intestinal incubation studies in rabbits (Hayashi-Bära, 1971) and *in vivo* jejunal perfusion studies in humans (Patil *et al.*, 1987). Grimble *et al.* (1988) orally administered 20 g of [ $^{14}\text{C}$ ]lactitol to adapted human subjects and collected 24-hr fecal and urine samples for 2 days and hourly  $\text{CO}_2$  samples for up to 10 hr followed by additional samples for up to 7 days. Two percent of the radiolabel was recovered in the urine, 6.5% in the feces, and 62% as  $^{14}\text{CO}_2$ . Peak  $^{14}\text{CO}_2$  occurred at 6 hr following ingestion indicating that it originated via bacterial fermentation in the colon. Confirmation of bacterial fermentation was provided by Wursch and Schweizer (1987), who orally administered 20 g of lactitol to adapted human subjects and detected large increases in breath hydrogen from bacterial fermentation. The fecal material was assumed to be  $^{14}\text{C}$ -assimilated into bacterial cells on the basis of increased dry fecal mass from rat lactitol feeding studies (Sinkeldam *et al.*, 1992).

### Maltitol

Mucosal homogenates of man, rat, and rabbits hydrolyze maltitol at a rate slower than maltose (Zunft *et al.*, 1983; Nilsson and Jagerstad, 1987). Human mucosal homogenates activities indicate a rate approximately 10% that of maltose hydrolysis, whereas in the rat, the hydrolysis rate increases to 30% that of maltose. Everted intestinal sacs from rabbits indicated glucose transport following incubation with maltitol, although the rate was slower than that from sucrose. Ileal perfusion in germ-free rats resulted in 19% disappearance of perfused maltitol, although stomach incubations resulted in 70% disappearance within 2 hr (Zunft *et al.*, 1983). Oral administration to humans results in elevations in blood glucose and insulin concentrations (Zunft *et al.*, 1983; Felber *et al.*, 1987; Akiba *et al.*, 1990), indicating that some hydrolysis and absorption of glucose from the small intestine occurs. Ileal perfusion of maltitol (57 g) indicated that 90% of the maltitol was hydrolyzed and absorbed from the small intestine (Beaugerie *et al.*, 1990). Langkilde *et al.* (1994) recovered 25% of a 15-g oral dose from the distal ileum of ileostomy patients. Following oral administration of [ $^{14}\text{C}$ ]maltitol to rats, Rennhard and Bianchini (1976) recovered 8% of the radiolabel in the feces, 5% in the urine, and 46% as breath  $^{14}\text{CO}_2$ . The fecal radioactivity was identified as short-chain fatty acids resulting from bacterial fermentation. Similar recoveries were found in humans. Akiba *et al.* (1990) recovered 14% of the radiolabel in the feces, 3% in the urine, and 56% as breath  $^{14}\text{CO}_2$  following oral administration of [ $^{14}\text{C}$ ]maltitol to human volunteers. Fermentation following oral administration was confirmed with the quantitation of significant amounts of breath hydrogen (Akiba *et al.*, 1990; Beaugerie *et al.*, 1991).

### Maltitol Syrup (Hydrogenated Glucose Syrups)

Maltitol syrups, formerly known as hydrogenated glucose syrups, consist primarily of maltitol (50–98%, anhydrous basis), oligomeric and polymeric polyols, and relatively small amounts of sorbitol (e.g.,  $\leq 8\%$ , anhydrous basis). Maltitol syrups are one member of the family of related polyols commonly referred to as hydrogenated starch hydrolysates. Other members of that family include sorbitol syrup ( $\geq 50\%$  sorbitol, anhydrous basis) and similar mixtures commonly referred to as HSH (not currently monographed) that are characterized by the presence of oligo- and polysaccharide-derived polyols as the major component. In that the metabolism of HSH products and maltitol syrups is substantively similar, information on their metabolism is discussed as a group within this section.

The composition can be designated by listing the percentage of each component following the name. Therefore, a maltitol syrup with the composition 6:52:42 is composed of 6% sorbitol, 52% maltitol, and 42% higher

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glucose homologs in which only the terminal glucose residue is hydrogenated. Similarly, an HSH syrup denoted as 10:8:82 contains 10% sorbitol, 8% maltitol, and 82% oligomeric and polymeric polyols. The metabolism of several different maltitol syrups, as well as HSH and maltitol, have been investigated and have been reviewed by Modderman (1993).

Dahlqvist and Telenius (1965) indicated that mucosal homogenates of rats and humans released 80% of the available glucose from an HSH preparation (10:8:82). Similar *in vitro* digestion studies with amylglucosidases with a range of maltitol syrup products, including an HSH preparation (6:24:70), maltitol syrup (6:52:42), and maltitol syrup (4:71:25), indicated release of glucose in direct proportion to the amount of higher polyol oligosaccharides present in the preparation (Leroy, 1978). Dahlqvist and Telenius (1965) indicated that 80% of the available glucose was absorbed in the small intestine after oral administration of an HSH preparation (10:8:82) to rats. Wheeler *et al.* (1990) indicated that an HSH preparation (14:8:78) and maltitol syrup (7:60:33) were less glycemic than glucose following oral administration to normal and diabetic subjects. Significant amounts of an HSH preparation (14:8:78) and maltitol syrup (1:60:33) reached the colon because increases in breath hydrogen were noted. Beaugerie *et al.* (1990) indicated that 90% of the higher oligosaccharides, 86% of the maltitol, and 64% of the sorbitol was absorbed from the small intestine following an oral dose (69 g) of maltitol syrup (7:53:41) in an ileum perfusion study of adapted human volunteers. Only trace amounts of glucose, sorbitol, or maltitol were detected in the feces, indicating that the material entering into the colon was fermented by the colon bacteria. Nguyen *et al.* (1993) orally administered maltitol syrup to human volunteers and noted increases in blood glucose, insulin, and C-peptide concentrations, although the responses were significantly lower than an equivalent amount of glucose.

### Isomalt

Several *in vitro* studies on the sensitivity of isomalt to the digestive enzymes of the small intestine have indicated that isomalt is relatively resistant to hydrolysis (Grupp and Siebert, 1978; Nilsson and Jagerstad, 1987). Recovery studies with ileal cannulated pigs (Van Weerden and Huisman, 1993a,b) and ileostomy patients (Kroneberg *et al.*, 1979; Langkilde *et al.*, 1994) administered isomalt confirm that a large portion of isomalt enters the colon and is not absorbed in the small intestine. Measurements of blood glucose and insulin concentrations following large oral doses of isomalt in healthy and diabetic humans indicated that isomalt did not increase these levels (Bachmann *et al.*, 1984; Thiebaud *et al.*, 1984). Its fermentation in the colon was confirmed by several studies in which breath

hydrogen was measured following oral administration (Fritz *et al.*, 1985; Beaugerie *et al.*, 1991; Lee *et al.*, 1994). Following oral administration to adapted rats, pigs, and humans, only trace amounts of isomalt or its hydrolysis products, sorbitol, mannitol, and glucose, could be found in feces (Musch *et al.*, 1973; Grupp and Siebert, 1978; Van Weerden and Huismann, 1993a).

Figdor *et al.* (1987) administered [ $^{14}\text{C}$ ]isomalt orally to fasted rats and recovered 12% of the radiolabel in the feces and 3.6% in the urine following 3-day collections and 48% in breath  $\text{CO}_2$  after 13 hr.

### Lactose

Several rat studies have demonstrated that only a portion of dietary lactose is absorbed from the small intestine. Dahlqvist and Thomson (1964) estimated that 60–75% of the orally administered lactose (2–4 g/kg body wt) was absorbed from the small intestine of Sprague–Dawley rats, whereas Kim *et al.* (1978) estimated that 57–68.5% of the orally administered lactose dose (30% of the diet) was absorbed from the small intestine of adapted male Holtzman rats.

In human subjects, Bond and Levitt (1976) indicated that relatively small doses of lactose (12.5 g) were completely absorbed in the small intestine (92–100%). Lactase-deficient subjects had lower absorptions ranging from 25 to 58%.

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#### APPENDIX D: COMPARATIVE ANATOMY, PHYSIOLOGY, AND PATHOLOGY OF THE NORMAL AND HYPER-/NEOPLASTIC ADRENAL MEDULLA IN RATS AND HUMANS

##### Basic Anatomy of the Adrenal Medulla

Mammalian species all have paired adrenal glands located in the perirenal fat adjacent to the kidneys (Hamlin and Banas, 1990; Tischler and Coupland, 1994). The adrenals of humans and rodents are composed of two tissues, the cortex and the medulla, which are developmentally, histologically, and functionally unrelated. The cortex, which comprises up to 90% of the weight of the adrenal gland, is mesodermal in origin and serves in the production of adrenocortical steroid hormones such as glucocorticoids, cortisone, and some adrenal androgens and estrogens (Hamlin and Banas, 1990; Capen *et al.*, 1991). The adrenal medulla of both rats and humans is derived from neuroectodermal stem cells and is sharply demarcated from the overlying cortical tissue, although this boundary may be irregular in outline, with cords of cortical tissue extending deep into the medullary tissue (Tischler and DeLellis, 1988a; Duprat *et al.*, 1990; Tischler and Coupland, 1994). The adrenal medulla is richly innervated by cholinergic preganglionic sympathetic endings from the splanchnic nerve. These nerve endings synapse directly with hormone-secreting medullary cells. In both rodents and humans, the adrenal medulla is essentially a sympathetic ganglion modified to be a neuroendocrine organ. The products of the medullary cells include several catecholamines that are important in the response to stress or crisis (Tischler and DeLellis, 1988a; Hamlin and Banas, 1990; Capen *et al.*, 1991).

The bulk of the adrenal medulla in rodents and in

humans is composed of chromaffin cells, the sites of synthesis and storage of catecholamines. Traditionally, these cells have been identified by their "chromaffin" reaction, or the formation of a brown color due to the oxidation of the catecholamine stores. Chromaffin cells in the young adult rat are typically arranged in nests and cords that can be highlighted by reticulin stains. Numerous vascular channels, chiefly capillaries, are also a significant feature in the medullary tissue. A small portion of the adrenal medullary volume, about 5%, is composed of myelinated and unmyelinated nerve fibers, with the remainder of the medullary volume composed of connective tissue elements.

In the rat, the development of catecholamine synthesis in the adrenal medulla has been described as proceeding through three stages (Verhofstad *et al.*, 1985; Tischler and DeLellis, 1988a; Hamlin and Banas, 1990). The first stage, ongoing through to about the 17th prenatal day, is characterized by the presence of norepinephrine and smaller amounts of dopamine and the lack of epinephrine (Tischler and DeLellis, 1988a). From the 17th prenatal day to about the 3rd day postpartum, epinephrine and norepinephrine are present in all medullary cells (Hamlin and Banas, 1990). At this time, the ratio of epinephrine to norepinephrine secretion increases to adult levels. Following the 3rd day postpartum, the medullary cells differentiate into cell types that predominantly secrete epinephrine or norepinephrine but not both (Tischler and DeLellis, 1988a; Coupland and Tomlinson, 1989; Hamlin and Banas, 1990; Tischler and Coupland, 1994).

Mechanisms that govern these developmental stages in the rat adrenal medulla have not been fully characterized (Tischler and DeLellis, 1988a) but are likely to involve adrenocortical hormones and nerve growth

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factors (Unsicker, 1989). These types of hormonal substances act interdependently to stimulate the maturation of the chromaffin cells, during which time the chromaffin cells differentiate into their various lineages or transdifferentiate into neural type cells. Based on experiments conducted by Tischler *et al.* (1994), it appears that neurally derived signals controlling catecholamine secretion and cell proliferation supersede the importance of growth factors and other humoral substances.

The pluripotent nature of developing chromaffin cells has been demonstrated *in vitro* (Unsicker *et al.*, 1978; Hoffman *et al.*, 1987; Herman *et al.*, 1991) and *in vivo* (Aloe and Levi-Montalcini, 1979). The plasticity of developing adrenal medulla chromaffin cells is dependent on the age and maturity of the cells, with chromaffin cells in adult rats being resistant to stimuli that can induce differentiation of the chromaffin cells in young rats (Tischler *et al.*, 1982; Doupe *et al.*, 1985; Tischler and DeLellis, 1988a).

In the rat, epinephrine and norepinephrine are largely stored in separate cells that can be distinguished ultrastructurally following fixation in glutaraldehyde and postfixation in osmium tetroxide (Brown *et al.*, 1971). Cell types with granules containing norepinephrine appear highly electron dense, whereas those with epinephrine containing granules are not as electron dense and are more finely particulate in appearance (Brown *et al.*, 1971; Tischler and DeLellis, 1988a; Hamlin and Banas, 1990; Capen *et al.*, 1991). Norepinephrine-containing cells in the rat are also slightly smaller than cells containing epinephrine, occupy about 20% of the medullary volume, and occur as clusters of cells within a background of epinephrine-containing cells (Tischler and DeLellis, 1988a; Hamlin and Banas, 1990; Capen *et al.*, 1991; Tischler and Coupland, 1994). In humans, norepinephrine- and epinephrine-containing cells are not segregated as they are in the rat (Brown *et al.*, 1971; Tischler and DeLellis, 1988a; Capen *et al.*, 1991; Tischler and Coupland, 1994). Human adrenal medullary chromaffin cells contain both norepinephrine and epinephrine within a single cell (Capen *et al.*, 1991).

Another possibly important histological difference between human and rat adrenal medullary tissue is the presence of a third cell type, the small granule containing cell (SGC) in the rat adrenal medulla. There is no clearly defined human counterpart for the SGC cell in rats. The SGC cell makes up less than 1% of the medullary tissue in the rat and is thought to be of chromaffin cell lineage (Coupland *et al.*, 1977; Coupland, 1989; Tomlinson and Coupland, 1990). The SGC cell is about the same size as typical chromaffin cells, but has smaller granules and secretory vesicles similar to those observed in neurons (Tischler and Coupland, 1994). As a result, morphologically SGC cells appear intermediate between chromaffin cells and ganglion

cells (Capen *et al.*, 1991). The functions of the SGC cells in the rat adrenal medulla are largely unknown.

The granules within the chromaffin cells of both humans and rodents are the sites of catecholamine synthesis (Tischler and DeLellis, 1988a; Hamlin and Banas, 1990; Capen *et al.*, 1991; Tischler and Coupland, 1994). Catecholamines comprise about 20% of the dry granule weight, with the remainder made up of acidic proteins (chromogranins), adenosine triphosphate, other nucleotides, neuropeptides (e.g., enkephalins and serotonin neuropeptide Y), glycosaminoglycans, and enzymes involved in the production and processing of catecholamine (Tischler and DeLellis, 1988a). Specific enzymes that have been identified include dopamine hydroxylase (synthesis of norepinephrine) and peptidases (neuropeptide processing) (Tischler and DeLellis, 1988a; Tischler and Coupland, 1994).

The functions of the acidic proteins (chromogranins) that compose the majority of the dry weight of chromaffin granules are not clear. Three classes of these proteins have been identified (chromogranins A-C) with the proportions of these classes showing clear species differences (Fischer-Colbrie and Frischenschlager, 1985; Winkler *et al.*, 1986; Schober *et al.*, 1987). In rats, chromogranin A predominates (Fischer-Colbrie and Frischenschlager, 1985), whereas in humans chromogranins A and B are present in about equal quantities (Schober *et al.*, 1987). The functions of the chromogranins are not clear. They may be trophic or regulating factors or peptide hormone precursors, may play roles in buffering or calcium binding (Winkler *et al.*, 1986; Tischler and DeLellis, 1988a), or may be hormone precursors (Winkler, 1993).

Secretory activity of the chromaffin cells is regulated by combinations of humoral and neurogenic signals (Tischler and DeLellis, 1988a; Tischler and Coupland, 1994). Neurogenic signals received via interactions of neurotransmitters, hormones (e.g., adrenocortical hormones), or other peptide hormones, such as vasoactive-intestinal peptide (Malhotra *et al.*, 1989) or pituitary adenylate cyclase activating peptide (PACAP) (Watanabe *et al.*, 1992), with their respective receptors, are transmitted into the interior of chromaffin cells via transduction pathways involving cAMP and activation of protein kinase A, phosphatidyl inositol and activation of protein kinase C, and calcium ions. The interaction of these pathways at the intracellular level is complex, with the degree of neuropeptide synthesis and release by the chromaffin cell being a product of the various signal transduction pathways (Doupe *et al.*, 1985; Tischler and DeLellis, 1988a; Unsicker, 1989; Tischler, 1989; Tischler and Coupland, 1994).

Secretion of the chromaffin cells following cholinergic or muscarinic receptor binding occurs as a result of calcium-mediated exocytosis (Trifaro *et al.*, 1985; Tischler and DeLellis, 1988a; Tischler, 1989; Burgoyne *et al.*, 1993). The neuronal activation of cholinergic syn-

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apses results in depolarization of the cell membrane and an influx of calcium ions through both the nicotinic receptor channel and the voltage-dependent calcium channels. Although the exact mechanism by which calcium influx results in exocytosis and epinephrine/norepinephrine secretion is unclear, one hypothesis suggests that the increased cytosolic calcium concentration results in the activation of a calcium-calmodulin-dependent protein kinase. The activation of this protein kinase has been suggested to result in dephosphorylation of a membrane-associated granule protein, which then causes destabilization of the cytoskeletal network and subsequent fusion of the granule membrane with the cell membrane and discharge of the granule contents (Trifaro *et al.*, 1985; Burgoyne *et al.*, 1993). In contrast to this cytoskeletal-based model of exocytosis is a model in which multiple proteins known as "SNAPS" and "SNARES" both dock the granule to the cell membrane and contribute to  $\text{Ca}^{2+}$ -dependent granule release (Söllner *et al.*, 1993). Recent evidence suggests that elements of both models are correct.

To summarize, the gross anatomy and embryology of the adrenal medulla are similar in most mammalian species, including rats and humans. There are, however, several important morphological, biochemical, and functional differences between human and rat adrenal medullary tissue. The key differences include

- Chromaffin cells in the rat differentiate into separate epinephrine- and norepinephrine-secreting cells, whereas in humans there are no clear distinctions between chromaffin cell types
- In the rat there is a third cell lineage present in the adrenal medulla, namely the small granule-containing cell, for which there is no clearly defined human counterpart
- The protein composition, specifically the chromogranin content, of the secretory granules contained within the chromaffin cells is significantly different between rats and humans.

#### *Response of Adrenal Medulla in Rats and Man to Mitogenic and Other Stimuli*

Once terminally differentiated, rat chromaffin cells were thought for years to be quiescent and relatively incapable of replication (Tischler and DeLellis, 1988a). However, in 1968, Malvadi *et al.* (1968) demonstrated that although mitotic activity of the chromaffin cells declines rapidly after birth in the rat (Jackson, 1919), small numbers of mature-appearing chromaffin cells are capable of cell division in the adult rat. Recent studies have confirmed this observation and show that both norepinephrine- and epinephrine-secreting chromaffin cells proliferate throughout life (Tischler *et al.*, 1988, 1989; Coupland and Tomlinson, 1989; Tischler and Coupland, 1994). Mitoses in SGC cells, however, are extremely rare (Coupland and Tomlinson, 1989).

Control of postnatal cell proliferation appears to be related to supra spinal innervation because newborn rats subject to spinal cord transection have shown reduced synaptic density and cell number in the adrenal medulla (Ross *et al.*, 1983). In contrast to the observations reported in the rats, chromaffin cell proliferation continues in humans for a prolonged period after birth, but then declines to very low levels and is almost never seen in routine histological sections of normal adult human adrenal medullary cells (Coupland, 1969; Tischler *et al.*, 1989).

The response of rat adrenal medullary tissue to specific mitogenic stimuli has been investigated in a number of studies. In one of the first studies, in response to a finding of increased frequency of pheochromocytomas in the male rat following oral administration of reserpine (an antihypertensive agent with neurological activity) in a 2-year cancer bioassay (Diener, 1988), Tischler *et al.* (1988) demonstrated that a short course of treatment with reserpine increased chromaffin cell proliferation in the rat. In a subsequent study, following 5 consecutive daily subcutaneous injections of reserpine (5–20 mg/kg body wt) to 9-month-old Long-Evans and Sprague-Dawley rats, Tischler *et al.* (1989) reported dose-dependent increases in the normalized chromaffin cell mitotic count. This effect was not observed following a single injection. In agreement with previous findings (Tischler *et al.*, 1988), mitoses in response to reserpine treatment were observed in both epinephrine- and norepinephrine-type chromaffin cells (Tischler *et al.*, 1989). The dividing reserpine-treated chromaffin cells were observed to exhibit varying degrees of secretory granule depletion. Given that the chromaffin cells that were dividing also showed the greatest secretory activity, and given that reserpine directly depletes catecholamine stores and causes increased activity of the splanchnic nerve stimulating the adrenal medulla, it would appear that the neurogenic signals stimulating the chromaffin cells following reserpine treatment also stimulate cell proliferation to meet the increased requirements for catecholamine secretion (Tischler *et al.*, 1989). In the Tischler *et al.* (1989) experiment, the magnitude of the increase in chromaffin cell proliferative activity in the different rat strains in response to reserpine were similar, although the Long-Evans rat has a higher spontaneous incidence of adrenal medullary tumors than the Sprague-Dawley rat (Tischler *et al.*, 1989). Tischler *et al.* (1989) also noted that although reserpine caused an increase in chromaffin cell proliferation *in vivo*, likely through neurogenic mechanisms, subsequent progression of adrenal medullary tumors may be partially dependent on hormonal influences because it has been demonstrated that adrenal medullary tumors lose their nerve supply (Tischler *et al.*, 1985). Hormonal substances that have been reported or postulated to affect the proliferation of rat chromaffin cells *in vitro* include insulin-like growth



factors (IGFs), notably IGF-1, which is regulated by circulating levels of pituitary growth hormone (Dahmer and Perlman, 1988a). One report has shown that hypophysectomy can potentially prevent the development of adrenal medullary tumors in a susceptible rat strain, whereas another has indicated that in PC12 rat pheochromocytoma cells, IGF-1 can induce increased rates of cell proliferation *in vitro* (Dahmer and Perlman, 1988b).

The role of innervation in the stimulation of spontaneous and reserpine-associated chromaffin cell proliferation was further investigated by Tischler *et al.* (1991). In this study, 5-bromo-2'-deoxyuridine incorporation was measured in the replicating nuclei of chromaffin cells of a group of F344 rats administered reserpine or a group of sham controls. The adrenals of all rats were unilaterally denervated. Denervation was reported to decrease the labeling of the chromaffin cells in control rats by four- or fivefold with norepinephrine- and epinephrine-type cells equally affected. Suppression of labeling was also observed in animals receiving reserpine in the diet (Tischler *et al.*, 1991). This finding suggests that innervation of the adrenal is critical for chromaffin cell proliferation and possibly a requisite for development of proliferative lesions.

In *in vitro* cell preparations, adult rat chromaffin cell proliferation is stimulated by phorbol ester PMA, a cholinergic innervation mimic capable of activating protein kinase C, and by cholera toxin (forskolin), a mimic of peptidergic innervation that acts by activating adenylate cyclase. In addition, the peptide neurotransmitter PACAP has been shown to be directly mitogenic at physiological concentrations (Tischler *et al.*, 1995a) and its mitogenic effects have been shown to be potentiated by indolidan, a phosphodiesterase inhibitor reported to cause adrenal tumors in rats.

In addition to neural-type signals, proliferation is stimulated by NGF, which may subsequently cause transdifferentiation of adrenal chromaffin cells into postmitotic neurons (Herman *et al.*, 1991; Tischler and Riseberg, 1993; Tischler *et al.*, 1993). NGF also has been demonstrated to be a potent inducer of proliferation and neuronal differentiation in adult rat chromaffin cells *in vitro* (Tischler *et al.*, 1993). Because inhibitors of protein kinase C, such as staurosporine, block the proliferative and differentiating effects of NGF, NGF may influence the development of rat chromaffin cells in part through mediation of the activity of protein kinase C (Herman *et al.*, 1991; Tischler *et al.*, 1993).

Cross-communication between mitogenic signal transduction pathways has been investigated by Tischler *et al.* (1994). Cholera toxin or forskolin, which are activators of adenylate cyclase, in cultures were found to cause an increase in the bromodeoxyuridine labeling index of adult F344 rat chromaffin cells. These agents were also reported to inhibit the mitogenic activity of NGF and PMA when incubated in combination

(Tischler *et al.*, 1994). Stimulation of mitotic activity was eliminated when potassium chloride or veratridine were added to the media to depolarize the chromaffin cells. Calcium channel activation by Bay K 8644 and the presence of adrenal cortical steroids (dexamethasone) were reported to also inhibit the mitogenic responses to inducers of adenylate cyclase and to NGF (Tischler *et al.*, 1994). The existence of multiple mitogenic signaling pathways, operating at different levels, with both inhibitory and stimulatory effects on mitogenic signaling is supported by the *in vitro* data on the specific effects of activators of protein kinases, adenylate cyclases, and calcium channel activators (Tischler *et al.*, 1994).

In summary, induction of mitotic activity of chromaffin cells following neuronal activation appears to be the result of a combination of the activation of protein kinase C, which follows acetylcholine binding to muscarinic cholinergic receptors (Malhotra *et al.*, 1989), and the activation of adenylate cyclase and protein kinase A, which follows the receptor binding of neuropeptides (Tischler *et al.*, 1985; Watanabe *et al.*, 1992). These second messenger systems also cross-communicate and act at different levels to regulate calcium mobilization and subsequent exocytotic secretion of catecholamines (Malhotra *et al.*, 1989).

Although mitogenic signaling mechanisms have been studied in the rat, fewer data are available for humans. Preliminary data indicate that the chromaffin cells of adult human medullary tissue are likely to be inherently less responsive to mitogenic stimuli than adult rat chromaffin cells (Tischler, 1992; Tischler and Riseberg, 1993). In an *in vitro* study comparing preparations of adult F344 rat chromaffin cells and chromaffin cells from human patients undergoing radical nephrectomy, but with otherwise normal adrenal function, Tischler (1992) and Tischler and Riseberg (1993) demonstrated that the addition of 100 ng/ml NGF or 50 nM TPA, followed by pulsing with bromodeoxyuridine, resulted in marked increases of labeled rat chromaffin cells compared to control media but not of human chromaffin cells. The presence of chromaffin cells was verified by staining to show the presence of tyrosine hydroxylase (Tischler, 1992; Tischler and Riseberg, 1993). Tischler (1992) and Tischler and Riseberg (1993) concluded that human chromaffin cells had an apparently lower responsiveness than rat chromaffin cells to mitogenic signals involving activation of protein kinase C. The results of preliminary unpublished *in vitro* studies, summarized in Tischler and Riseberg (1993), provide additional evidence to indicate that the spontaneous proliferation capacity of human chromaffin cells is low as measured by the lack of significant quantities of PCNA and the lack of effects of adenylate cyclase activators on the mitogenic activity of human chromaffin cells. Tischler and Riseberg (1993) also suggested that a difference in sensitivity to mitogenic stimuli could

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contribute to the lower frequency of adrenal medullary lesions in humans compared to many rat strains. It is also interesting to note that the mouse, like humans, has a low spontaneous incidence of proliferative lesions of the adrenal medulla; also, similar to humans, the chromaffin cells do not respond to nerve growth factor, activators of adenylate cyclase, or activators of protein kinase C, all of which are mitogenic to rat chromaffin cells (Tischler and Sheldon, 1995).

In summary, there are several important interspecies differences between rats and humans in the response of the adrenal medullary tissue to mitogenic stimuli. The key differences include

- Catecholamine-secreting chromaffin cells of the rat adrenal medulla have been demonstrated to be capable of proliferation following postnatal differentiation; however, in humans, mitoses in mature chromaffin cells are very rare
- Proliferation of chromaffin cells in the adult rat has been shown *in vitro* to be induced by mitogenic agents, such as FGF, NGF, and forskolin, which activate protein kinases or adenylate cyclases, whereas the chromaffin cells of humans are resistant to these mitogenic stimuli.

In conclusion, *in vitro* the chromaffin cells of the human appear to be inherently less responsive than those of the rat to mitogenic stimuli. This also reflects the overall lower rates of spontaneous cell proliferation and lower incidence of spontaneous proliferative lesions in the human adrenal medulla. Chromaffin cells of the rat appear to represent an inappropriate model to assess the potential effects of chemicals on human chromaffin cells.

#### *Proliferative Lesions of the Adrenal Medulla in Rats and Man*

**Rats.** The adrenal medulla is a common site for the occurrence of proliferative lesions in the rat. In the scientific literature, two general types of proliferative lesions have been described (Thompson *et al.*, 1981; Tischler and DeLellis, 1988b). These are hyperplasia, either diffuse or hyperplastic nodules, and frank adrenal medullary tumors (pheochromocytoma), either benign or malignant (Thompson *et al.*, 1981; Tischler and DeLellis, 1988b; Hamlin and Banas, 1990; Capen *et al.*, 1991).

Hyperplasia of the adrenal medulla in the rat is characterized by aggregates of medullary cells with minimally altered cellular arrangement and cytological features; however, the arrangement of cells in clusters or pockets is more obvious. The cells tend to be slightly enlarged with round vesicular nuclei or slightly smaller than normal but with hyperchromatic nuclei. Also, areas of focal or diffuse hyperplasia may be somewhat more basophilic than the surrounding medullary tis-

sue. Compression of the surrounding medullary tissue, or the juxtacortical tissue, an area in which focal hyperplasia often arises, is not apparent (Thompson *et al.*, 1981; Tischler and DeLellis, 1988b; Hamlin and Banas, 1990; Tischler and Coupland, 1994).

Areas of hyperplasia may enlarge to form small nodules. These are generally well-demarcated masses that contain cells that are slightly smaller, have less cytoplasm, are more basophilic, and have greater numbers of pleomorphic nuclei than cells in hyperplastic tissue (Thompson *et al.*, 1981; Tischler and DeLellis, 1988b; Hamlin and Banas, 1990; Tischler and Coupland, 1994). As adrenal medullary nodules enlarge, they tend to compress the surrounding tissue. There is a progressive loss of innervation and displacement of reticulin as the nodules enlarge (Thompson *et al.*, 1981; Tischler and DeLellis, 1988b; Hamlin and Banas, 1990; Tischler and Coupland, 1994). Compared to normal adrenal medullary cells, and those in hyperplastic tissue, the cells contained in nodules show a homogenous population of granules that are small, sparse, and electron dense. As a result, the cells contained within medullary nodules are norepinephrine containing with few, if any, cells containing epinephrine (Gillman *et al.*, 1953; Tischler *et al.*, 1985, 1990; FASEB, 1986; Tischler and DeLellis, 1988b; Hamlin and Banas, 1990). Histochemical staining of adrenal medullary tumors with markers for catecholamine synthesis is less intense than in surrounding normal or hyperplastic medullary tissue (Tischler *et al.*, 1990). Proliferative lesions in the rat adrenal medulla usually also produce hormones generally associated with norepinephrine-secreting cells. These hormones may include dopamine, neurotensin, and neuropeptide Y (Thompson *et al.*, 1981; Tischler *et al.*, 1985; Manger *et al.*, 1985).

The morphological continuum that is observed in the progression of hyperplasia to adrenal medullary nodules makes any classification scheme somewhat arbitrary. The benign or malignant nature of neoplastic adrenal medullary nodules in the rat has historically been assigned on the basis of increased nuclear/cytoplasmic ratio, cellular pleomorphism and atypia, increased mitotic index, hemorrhage, necrosis, invasion of the capsule, the presence of metastases, and size (Thompson *et al.*, 1981; Hamlin and Banas, 1990; Duprat *et al.*, 1990; Capen *et al.*, 1991; Tischler and Coupland, 1994). Although capsular invasion is the major criterion for malignancy in toxicologic pathology, invasion is not predictive of proclivity to metastasize, and metastases are rare. In human pathology, it is recognized that aside from the presence of metastases, there are no unambiguous features with which to determine the malignant nature of a tumor (Tischler *et al.*, 1990; Hamlin and Banas, 1990).

Functionally, there are no qualitative differences that can be used to distinguish focal hyperplasia or hyperplastic nodules from nodules that have been his-

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torically termed "pheochromocytoma" (Tischler *et al.*, 1990). In an immunocytochemical analysis of rat adrenal medullary lesions classified as either pheochromocytoma or hyperplastic nodules, Tischler *et al.* (1990) reported that 16 of 17 pheochromocytomas, and all hyperplastic nodules, positively stained (although somewhat less than in surrounding normal medullary tissue) for tyrosine hydroxylase and dopamine  $\beta$ -hydroxylase, indicating that both of these proliferative lesions were capable of synthesizing norepinephrine. Also, both the hyperplastic nodules and the pheochromocytomas in the Tischler *et al.* (1990) study failed to stain for markers that would indicate an ability to produce epinephrine. This observation was consistent with the results of several *in vivo* investigations and *in vitro* work with a cultured rat pheochromocytoma cell line (PC12 cells), which demonstrated that adrenal medullary tumors in the rat were incapable of producing epinephrine (Gillman *et al.*, 1953; Tischler *et al.*, 1985, 1990). As a result of their experiments, Tischler *et al.* (1990) concluded that qualitative functional differences could not be used to differentiate lesions considered to represent pheochromocytoma from lesions classified as hyperplastic nodules.

The lack of epinephrine-producing cells in the adrenal medullary lesions of rats is perplexing in that both epinephrine- and norepinephrine-producing cells have been shown to proliferate in response to mitogenic stimuli (Tischler *et al.*, 1988, 1989; Coupland and Tomlinson, 1989; Tischler and Coupland, 1994). The causal factors involved in the progressive shift to norepinephrine-producing cells in the development of rat adrenal medullary tumors are unclear. It has been suggested that because hyperplastic nodules and tumors often develop in the juxtacortical area, an area in which the concentration of small granule containing cells is the greatest, the small granule containing cell may act as a precursor (Tischler and DeLellis, 1988b; Tischler and Coupland, 1994) from which only the cells of the norepinephrine-secreting lineage develop into tumors, even though other lineages have the capacity to proliferate in response to mitogenic stimuli. Alternatively, and perhaps more likely, the noradrenergic phenotype occurs secondarily to neoplastic transformation.

Adrenal medullary lesions in the adult rat are usually not hyperfunctional; that is, they generally do not secrete excessive amounts of catecholamines or produce symptoms such as increased blood pressure (Thompson *et al.*, 1981; Roe and Bär, 1985; FASEB, 1986; Tischler and DeLellis, 1988b). This statement, however, is only a generalization because there are several reports of adrenal medullary neoplasms in rats that are associated with increased plasma catecholamine levels, increased blood pressure, and clinical symptoms (Warren and Chute, 1971; Roe and Bär, 1985; Manger *et al.*, 1985).

Over the past several decades, a number of chemical

agents have been reported to be associated with an increased incidence of proliferative lesions of the adrenal medulla in the rat, especially in males. Several general mechanisms appear to be involved in the development of adrenal medullary proliferative lesions. Substances, such as growth hormone, estrogen, antithyroidal drugs, neuroleptics and alloxan, which disturb the hypothalamic-pituitary axis have been associated with the development of pituitary lesions (Tischler and DeLellis, 1988b; Hamlin and Banas, 1990; Duprat *et al.*, 1990; Capen *et al.*, 1991). Compelling experimental evidence has been provided to suggest that pituitary hormones play an important role in the development of adrenal medullary lesions (Manger *et al.*, 1982). Other chemicals, such as reserpine and nicotine, which stimulate the autonomic nervous system, thereby increasing neural stimulation of the adrenal medulla, are also associated with an increased incidence of proliferative lesions (Tischler and DeLellis, 1988b; Diener, 1988). It is apparent that hormonal and neuronal regulation, both known to be involved in the stimulation of catecholamine secretion and chromaffin cell proliferation (Malhotra *et al.*, 1989; Tischler *et al.*, 1994), are also involved, likely in an overlapping manner, in the development of proliferative lesions in the rat adrenal medulla.

One of the most striking observations regarding proliferative lesions in the adrenal medulla of rats is the variable, but often high, spontaneous incidence at which they occur (Thompson *et al.*, 1981; Strandberg, 1983; Roe and Bär, 1985; Tischler *et al.*, 1985, 1989; Tischler and DeLellis, 1988b; Hamlin and Banas, 1990; Duprat *et al.*, 1990; Capen *et al.*, 1991). In addition, the spontaneous incidence of these lesions appears to show a strong strain-dependent effect (Thompson *et al.*, 1981; Tischler and DeLellis, 1988b; Hamlin and Banas, 1990; Capen *et al.*, 1991). Several factors have been reported to influence the spontaneous incidence rates of adrenal medullary lesions in rats of different strains including rats being reared by different breeders, fed various diets, sacrificed after varying lengths of time, and examined by different pathologists employing differing criteria by which to classify the observed adrenal medullary lesions. Despite these limitations, it is clear that some strains of rats appear to be relatively resistant to the development of tumors of the adrenal medulla (e.g., Holtzman and Osborne-Mendel rats), whereas in other strains these tumors arise frequently (e.g., Wistar and Long-Evans rats). Furthermore, there are clear age-related increases in incidence in all rat strains (Capen *et al.*, 1991), with most rat strains also showing a trend for males to be more susceptible to the development of these lesions. Table D1 presents the average spontaneous incidence of pheochromocytomas reported to occur in several rat strains used in bioassays of 2 years or greater duration.

The actual incidence of adrenal medullary tumors in

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**TABLE D1**  
**Spontaneous Incidence of Adrenal Medullary Tumors in Various Rat Strains**

Rat strain	Incidence (%)		Reference
	Males	Females	
Charles River-CD	109/1211 (9.0)	24/1204 (2.0)	Sher <i>et al.</i> (1982)
F344 (NTP)	158/1794 (8.8)	55/1754 (3.1)	Goodman <i>et al.</i> (1979)
F344	37/459 (8.1)	9/459 (2.0)	Sher <i>et al.</i> (1982)
Holtzman	4/806 (0.5)	3/618 (0.5)	Schardien <i>et al.</i> (1968)
F844	76/448 (17.0)	14/450 (3.1)	Sher <i>et al.</i> (1982)
Osborne-Mendel (NTP)	21/975 (2.2)	9/970 (0.9)	Goodman <i>et al.</i> (1980)
Sprague-Dawley (CIBA-GEIGY)	121/578 (20.9)	36/585 (6.2)	McMartin <i>et al.</i> (1992)
Wistar [BOR:WISW (SPF Cpb)] (30 month)	131/445 (29.4)	16/447 (3.6)	Bomhard (1992)
Wistar [BOR:WISW (SPF Cpb)] (24 month)	133/1230 (10.6)	27/1242 (2.1)	Bomhard and Rinke (1994)
Wistar (Lobund)	46/66 (69.2)	No data	Pollard and Luckert (1989)

any given study is subject to considerable variation. For example, the spontaneous incidence of adrenal medullary tumors has been reported to range up to 25–30% in F344 rats (Solleveld *et al.*, 1984), 31% in Sprague-Dawley rats (Tannenbaum *et al.*, 1962), 38% in Long-Evans rats (Tischler *et al.*, 1985), and up to 86% in Wistar rats (Gillman *et al.*, 1953). The incidence of hyperplasia of the adrenal medulla of the rat, like the incidence of adrenal medullary tumor, is variable and can be present at a significant incidence rate (see Table D1) (Crain, 1958; Goodman *et al.*, 1979, 1980; Thompson *et al.*, 1981; McMartin *et al.*, 1992).

**Humans.** The gross appearance of proliferative lesions of the adrenal medulla in humans is similar to that of rats. Diffuse and nodular hyperplasia of the adrenal medulla occur rarely in man and tend to be observed in humans with a familial disposition to the development of pheochromocytoma as part of multiple endocrine neoplasia syndromes (Carney *et al.*, 1975; DeLellis *et al.*, 1976; Thompson *et al.*, 1981; R  e and B  r, 1985; Tischler *et al.*, 1985; Duprat *et al.*, 1990). Histopathologically, the chromaffin cells contained within proliferative lesions of the human adrenal medulla show variability in their size, with some cells within the lesion appearing larger and others appearing smaller than the surrounding cells in the normal adrenal medullary tissue. In humans, chromaffin cells of proliferative lesions also tend to show no consistent granule morphology (Thompson *et al.*, 1981; Tischler *et al.*, 1985; FASEB, 1986; Tischler and DeLellis, 1988b). Generally, chromaffin cells within proliferative lesions contain abundant stores of epinephrine and stain strongly for markers of epinephrine synthesis (Tischler *et al.*, 1985; Hamlin and Banas, 1990; Tischler and Coupland, 1994).

Proliferative lesions of the human adrenal medulla, in keeping with the observation of extensive chromaffin staining, are usually functional in that they release catecholamines, often in excessive amounts that may

result in cardiovascular symptoms (Thompson *et al.*, 1981; Manger *et al.*, 1985; FASEB, 1986; Tischler and DeLellis, 1988b).

Although proliferative lesions of the adrenal medulla are a common feature of certain of the multiple endocrine neoplasia syndromes (DeLellis *et al.*, 1986; Caruso *et al.*, 1991) and have a genetic basis, no substances are known that are capable of inducing proliferative lesions, including pheochromocytoma, of the adrenal medulla in humans. The lack of inducibility may largely be related to the lack of susceptibility of humans to the spontaneous development of these lesions.

The incidence of proliferative lesions of the adrenal medulla in humans is considerably lower than that reported in rats. Based on the results of autopsy reports, the incidence of pheochromocytoma in the general human population has been estimated to range from 0.005 to 0.1% (Minno *et al.*, 1954; von Schlegel, 1960; Symington, 1969; Manger *et al.*, 1985). Most series favor the lower figure. Based on the information available, the spontaneous incidence rate of adrenal medullary tumors in humans would appear to be from near 100- to 2000-fold lower than that in rats. This striking difference in the background incidence of adrenal medullary lesions may be linked to the inherently greater sensitivity of the rat adrenal to mitogenic stimuli (Tischler and Riseberg, 1993) or could be related to reported functional and morphological differences between rat and human adrenal medullary tissue. It is of interest to note that in the mouse, as in humans, the incidence of spontaneous lesions in the adrenal medullary tissue is low compared to that of the rat (Capen *et al.*, 1991). This observation is in accordance with the report that both human (Tischler and Riseberg, 1993) and mouse chromaffin cells (Tischler and Sheldon, 1995; Tischler *et al.*, 1995b) are resistant to stimuli that are mitogenic to rat chromaffin cells and provides additional evidence to suggest that the high spontaneous incidence of adre-



nal medullary lesions in rats may be related to rat-specific sensitivity to mitogenic stimuli.

### Summary

In summary, proliferative lesions of the adrenal medulla in humans share several characteristics with lesions observed in rats. For example, diffuse and nodular hyperplasia of a type grossly similar to that reported in rats has been observed in humans with a familial disposition to the development of pheochromocytoma (Carney *et al.*, 1975; DeLellis *et al.*, 1976; Thompson *et al.*, 1981; Roe and Bär, 1985; Tischler *et al.*, 1985; Duprat *et al.*, 1990).

However, there are a number of important morphological and functional differences, many of which may be significant in a regulatory context, between the proliferative lesions observed in rats in toxicology studies and those reported to occur in humans. These include

- In humans, the cells contained within the proliferative lesions can be either smaller or larger than normal chromaffin cells, generally have no consistent granule morphology, and often contain abundant stores of epinephrine (Thompson *et al.*, 1981; Tischler *et al.*, 1985; FASEB, 1986; Tischler and DeLellis, 1988b). This contrasts sharply to observations in rats indicating that the proliferative lesions contain cells that are smaller than normal chromaffin cells, have small secretory granules, and show little staining for the presence of epinephrine synthesizing ability (Tischler *et al.*, 1985, 1989; Tischler and DeLellis, 1988b; Hamlin and Banas, 1990; Tischler and Coupland, 1994).

- Proliferative lesions in rats generally do not appear to be hyperfunctional, whereas in humans, proliferative lesions in the adrenal medulla often present clinical symptoms of catecholamine excess (Thompson *et al.*, 1981; FASEB, 1986; Tischler and DeLellis, 1988b). This distinction is not clearcut (Tischler and DeLellis, 1988b), however, because there are reports of symptomatic lesions in the New England Deaconess Hospital strain of rat and there are also reports of the finding of incidental, nonsymptomatic pheochromocytomas in humans at autopsy (Bravo *et al.*, 1979; Sutton *et al.*, 1981).

- In the rat, the spontaneous incidence of proliferative lesions of the adrenal medulla, including diffuse and focal hyperplasia, as well as frank tumor, is considerably greater and shows much more variability than similar lesions in the normal human population. This difference may be most important because it may relate to the sensitivity of rat chromaffin cells to mitogenic stimuli or to morphological and functional differences between rat and human chromaffin cells.

Based on the data available, it is apparent that significant morphological, functional, and etiological differences between rats and humans exist in regard to

the nature of proliferative lesions that occur in the adrenal medulla.

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**Memorandum**

Date September 28, 2001

From Consumer Safety Officer

Subject GRN 000059

To Administrative File, GRN 000059

This memorandum summarizes the notice dated September 11, 2000, that Grain Processing Corporation (GPC) and SPI Polyols, Inc. (SPI) jointly submitted in accordance with the agency's proposed regulation, proposed 21 CFR 170.36 (62 FR 18938; April 17, 1997; Substances Generally Recognized as Safe (GRAS)). The Office of Food Additive Safety (OFAS; formerly the office of Premarket Approval) received the notice on October 4, 2000, and designated it as GRN 000059.

The subject of the notice is hydrogenated starch hydrolysate (HSH). The notice informs FDA of the view of GPC and SPI (GPC/SPI) that HSH is GRAS, through scientific procedures, for use as a flavoring agent and adjuvant, formulation aid, humectant, processing aid, stabilizer and thickener, surface-finishing agent and texturizer in hard candy, soft candy, chewing gum, bakery products and ice cream.

As part of its notice, GPC/SPI include the report of Dr. Robert Lindhardt (GPC/SPI's consultant) who compared the HSH product manufactured by GPC/SPI to two other HSH products, one produced by Roquette Corporation (Roquette) and another produced by Lonza, Inc. GPC/SPI consider Dr. Lindhardt to be qualified by scientific training and experience to perform such an evaluation.

This memorandum summarizes the data and information that GPC/SPI describe to support their view that the intended use of HSH is GRAS. In general, this memorandum provides an abbreviated citation to references that are provided in the notice. Unless otherwise specified, the complete citation is available in the notice.

**Identity and Composition of HSH**

HSH (CAS Reg. No. 68425-17-2) is a concentrated, aqueous solution that contains sorbitol (0 to 10 percent), maltitol (0 to 10 percent), hydrogenated tri- to hexasaccharides (5 to 35 percent) and hydrogenated polysaccharides (greater than 50 percent on a dry product basis). Other names for HSH include malto-oligosaccharides, hydrogenated glucose syrup, and hydrogenated glucose solids. The components of HSH belong to a group of substances commonly called sugar alcohols or polyols.

**Method of Manufacture and Specifications**

In the manufacturing process for HSH, edible starch is hydrolyzed using alpha-amylase and acid. The starch hydrolysate is hydrogenated using Raney nickel as a catalyst. The product of the hydrogenation reaction is filtered, demineralized on ion-exchange resins, and concentrated to 50% (or greater) syrup or dried to a powder of approximately 5% moisture. GPC/SPI provide specifications for HSH, including a specification for heavy metals (as lead) of 0.1 milligrams per kilogram (0.1 parts per million).

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**Intended Conditions of Use and Estimated Dietary Exposure**

GPC/SPI's product will be used as flavoring agent and adjuvant, formulation aid, humectant, processing aid, stabilizer and thickener, surface-finishing agent, and texturizer in a wide variety of food products. GPC/SPI describe uses of currently marketed HSH as follows:

Food Type	Current Maximum Use Level	
	Liquid form	Dry powder
Hard candy	99%	60%
Soft candy	90%	50%
Chewing gum	45%	45%
Bakery Goods	35%	25%
Ice Cream	20%	15%

GPC/SPI estimates potential daily consumption of its HSH product based on the following conditions of use:

Food	HSH Level	Daily HSH Consumption (grams/person/day)
Confections*	10 - 100%	0.45 - 4.99
Cookies**	0 - 25%	0 - 2.72
Cakes**	0 - 10%	0 - 1.36
Sweet goods**	0 - 10%	0 - 1.81
Donuts**	0 - 2%	0 - 4.08
Carbonated Soft Drinks*	0 - 1%	0 - 4.08
Ice cream***	0 - 15%	0 - 5.89

\*USDA/Economic Research Service and U.S. Department of Commerce, Per capita Consumption '97

\*\* U.S. Census Bureau 1997 Economic Census

\*\*\*Prepared Foods July 1998

**Comparison of HSH Produced by GPC/SPI to HSH Produced by Roquette or by Lonza**

GPC/SPI compare their HSH product to an HSH product manufactured by Roquette Corporation (Roquette). Roquette's product, which is also known as Lycasin 80/55, is the subject of a filed GRAS affirmation petition, GRP 3G0286. GPC/SPI also compare their HSH product to an HSH product manufactured by Lonza, Inc. (Lonza). Lonza's product is the subject of a filed GRAS affirmation

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petition, GRP 5G0304. According to the report of GPC/SPI's consultant, all three HSH products are derived from edible starch using similar methods of manufacture, although GPC/SPI's hydrogenation step uses milder conditions than those of Roquette and Lonza. Qualitatively, the identified components of all three HSH products are the same; quantitatively, the three products contain differing amounts of each of these components. The quantitative composition of GPC/SPI's product more closely resembles that of Lonza's HSH than of Roquette's HSH, but has a somewhat higher theoretical molecular weight than that of Lonza's product.

To substantiate their view that the intended use of the HSH prepared by GPC/SPI is GRAS, GPC/SPI refer to Roquette's and Lonza's GRAS affirmation petitions. In each petition, the GRAS determination for the petitioned substance was based on scientific procedures; in each petition, the petitioner reported that a GRAS panel composed of members considered to be qualified by scientific training and experience to evaluate the safety of substances added to food had evaluated the data and information in the petition. Both GRAS panels considered the respective HSH product to be GRAS.

### **Studies**

In GPC/SPI's view, data and information in GRP 3G0286 (Roquette's petition) demonstrate that Lycasin 80/55 is broken down (96 percent) into sorbitol, glucose, and maltitol within the first 2 hours. Within 7 hours, 95 percent of maltitol is broken down into glucose and sorbitol. Of the remaining maltitol, 50 percent is excreted in the feces, 25 percent is excreted in the urine, and the rest remains in the blood stream. After 12 hours, maltitol blood levels drop to zero; there is no accumulation in the other tissues and organs of the body.

In GPC/SPI's view, data and information in GRP 5G0304 (Lonza's petition) demonstrate laxation effects from exposure to Lonza's HSH product. Based upon comparisons of the carbohydrate profile of Lonza's product to that of GPC/SPI's product, GPC/SPI conclude that the acceptable range of consumption for their HSH product is 100g/day.

GPC/SPI discuss an unpublished acute oral toxicity study that showed that the LD<sub>50</sub> for the HSH prepared by GPC/SPI is greater than 10 g/kg. In GPC/SPI's view, data and information in Roquette's petition demonstrate that an oral dose of 5-15 g/kg of Lycasin 80/55 in rats and dogs produces no toxicologically meaningful effects beyond those that can be accounted for by the presence of sorbitol.

GPC/SPI include a list of published articles on carcinogenicity and mutagenicity and a copy of a report of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (Ref. 1; the 46<sup>th</sup> JECFA report). According to the 46th JECFA report, published literature, including long-term studies in animals, demonstrates that high levels of polyols, such as those that occur in HSH, induced adrenal medullary hyperplasia and neoplasia in rats but not in mice or dogs. In addition, studies conducted in humans on safety and metabolism did not suggest any significant adverse effects. In its report, JECFA concludes that polyols are nonmutagenic and that the occurrence of adrenal medulla lesions in rats fed polyols and lactose is a species-specific phenomenon that is not relevant to the toxicological evaluation of polyols for humans.

### **Conclusions of Roquette's GRAS Panel on Roquette's HSH (Lycasin 80/55)**

GPC/SPI describe the deliberations of Roquette's GRAS panel, which was composed of past members of the Select Committee on GRAS Substances of the Federation of American Societies for Experimental Biology. Roquette's GRAS panel reviewed the reports and documents contained in GRP 3G0286 and concluded that Lycasin does not demonstrate a hazard to the public when used at levels and in the

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manner currently practiced at that time or that might reasonably be expected in the future. Roquette's GRAS panel asserted that Lycasin 80/55 is nonmutagenic, nonclastogenic, and produces no significant toxic effects on reproduction but may produce a laxative effect at an intake level of approximately 100 g per day.

#### **Rulemaking to authorize a health claim**

The ingredient HSH was one of several food ingredients that were the subject of a health claim petition about the association between sugar alcohols and the nonpromotion of dental caries (60 FR 37507 at 37509, July 20, 1995; the health claim proposal). Under 21 CFR 101.14(b)(3)(ii), a person who petitions FDA to authorize a health claim about the association between a food ingredient and a disease must demonstrate, to FDA's satisfaction, that the use of the ingredient, at levels necessary to justify a claim, is safe and lawful under the applicable food safety provisions of the Federal Food, Drug, and Cosmetic Act. Thus, as part of the rulemaking associated with the health claim petition, FDA evaluated whether the health claim petitioner had demonstrated, to the agency's satisfaction, that HSH is safe and lawful.<sup>1</sup> In a final rule that authorized this health claim (61 FR 43433 at 43436; August 23, 1996; the health claim final rule), FDA concluded that the petitioner had satisfied the requirements of 21 CFR 101.14(b)(3)(ii). The regulation that resulted from this rulemaking authorizes a health claim on foods, such as confectioneries and chewing gum, that do not contain fermentable carbohydrates (21 CFR 101.80). The regulation also authorizes a health claim on foods that contain fermentable carbohydrates in addition to sugar alcohols, provided that the mixture does not lower plaque pH below 5.7.<sup>2</sup>

GPC/SPI cite the 46th JECFA report, which discusses published studies in animals that demonstrate that high levels of polyols, such as those that occur in HSH, induced adrenal medullary hyperplasia and neoplasia in rats but not in mice or dogs. In its report, JECFA concludes that polyols are nonmutagenic and that the occurrence of adrenal medulla lesions in rats fed polyols and lactose is a species-specific phenomenon that is not relevant to the toxicological evaluation of polyols for humans. The JECFA report does not address other effects noted in published studies, such as the issue of Leydig cell tumors in rats fed high doses of the polyol lactitol (58 FR 47746, September 10, 1993; filing notice for a petition to affirm the GRAS status of lactitol, GRP 2G0391), or data from studies on mannitol that demonstrate a significant incidence of benign thymomas, and an abnormal growth of thymus gland tissue, in female rats fed mannitol (59 FR 64207; December 13, 1994; filing notice for food additive petition FAP 4A4412). Given issues such as these, in the health claim proposal FDA stated that a preliminary review of the available data and information had revealed significant evidence supporting the safety of these substances, but that some concerns about the safety of sugar alcohols do exist. Based on the totality of the evidence available at the time of the health claim proposal, FDA tentatively concluded not to challenge the position of the health claim petitioners that the use of the enumerated sugar alcohols is safe and lawful (60 FR 37507 at 37509). In the health claim final rule, following its analysis of comments

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<sup>1</sup>The health claim petition included several polyols in addition to HSH, such as sorbitol, lactitol, mannitol, maltitol, maltitol syrups, and isomalt. The GRAS status of these polyols is not the subject of GRN 000059.


<sup>2</sup>As discussed in the sugar alcohol proposal, the development of dental caries is the result of an interaction between sugars (and other fermentable carbohydrates) and oral bacteria in a suitable environment. Microorganisms in dental plaque metabolize available dietary sugars, producing acid and sticky polysaccharides that adhere to the tooth as plaque. Acid produced from rapid and complete fermentation of sugars creates an acid environment within the plaque, characterized by a pH of usually less than 5.0, that is capable of demineralizing tooth enamel and causing a carious lesion.

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regarding this tentative conclusion, FDA reiterated this view.

**GPC's Request that OFAS cease to evaluate GRN 000059**

In a letter dated September 14, 2001, GPC asked that OFAS cease to evaluate GRN 000059. Given this request, we ceased to evaluate GRN 000059, effective September 25, 2001, the date that we received GPC's letter.

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Linda S. Kahl, Ph.D.

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