

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.

GRN No. ¹	Description	FDA's Disposition of GRN	Total Pages	Page # in Main File
	Index		2	1
	NRDC's FOIA Request – October 11, 2013		9	3
	FDA's Confirmation of receipt -		1	12
	FDA's 1 st Response – January 16, 2014		1	13
	FDA's 2 nd Response – March 19, 2014		1	14
	FDA's 3 rd Response – March 19, 2014		1	15
GRN-1	Soy isoflavone extract	Nov 3, 1998 At notifier's request, FDA ceased to evaluate the notice	809	See GRN-1 File ²
GRN-35	Hempseed oil	Aug 24, 2000 Notice does not provide a basis for a GRAS determination	18	16
GRN-36	Chromium picolinate; <i>Ginkgo biloba</i> leaf extract; and Ginseng extract	Apr 10, 2000 At notifier's request, FDA ceased to evaluate the notice	15	34
GRN-37	Whey protein isolate and dairy product solids	Apr 21, 2000 FDA has no questions	6	49
GRN-59	Hydrogenated starch hydrolysate	Sep 24, 2001 At notifier's request, FDA ceased to evaluate the notice	333	See GRN-59 File ³
GRN-66	Milk thistle extract	Apr 23, 2001 Notice does not provide a basis for a GRAS determination	56	55
GRN-150	Glucosamine hydrochloride prepared from chitin obtained from <i>Aspergillus niger</i>	Sep 9, 2004 At notifier's request, FDA ceased to evaluate the notice.	77	111
GRN-224	trans-Resveratrol	Aug 1, 2007 At notifier's request, FDA ceased to evaluate the notice	5	183
GRN-225	Catechins from green tea extract	Nov 26, 2007 At notifier's request, FDA ceased to evaluate the notice / Resubmitted as GRN No. 259	13	188
GRN-257	<i>gamma</i> -Amino butyric acid	Dec 22, 2008 At notifier's request, FDA ceased to evaluate the notice	12	201

GRN No. ¹	Description	FDA's Disposition of GRN	Total Pages	Page # in Composite File
GRN-262/ GRN-263/ GRN-264	Sweet lupin protein / Sweet lupin fiber / Sweet lupin flour	Dec 16, 2008 At notifier's request, FDA ceased to evaluate the notice Dec 16, 2008 At notifier's request, FDA ceased to evaluate the notice Dec 16, 2008 At notifier's request, FDA ceased to evaluate the notice	82	213
GRN-295	Aqueous extract of <i>Emblica officinalis</i>	Nov 23, 2009 At notifier's request, FDA ceased to evaluate the notice. Resubmitted as GRN- 322	19	295
GRN-322	Aqueous extract of <i>Emblica officinalis</i>	Jun 16, 2010 At notifier's request, FDA ceased to evaluate the notice / Resubmission of GRN- 295 , resubmitted as GRN- 483 (which is pending)	8	314
GRN-324	Heat-killed <i>Lactobacillus plantarum</i>	July 7, 2010 At notifier's request, FDA ceased to evaluate the notice	94	322
GRN-340	Theobromine	Nov 10, 2010 At notifier's request, FDA ceased to evaluate the notice	215	See GRN-340 File ⁴
GRN-362	Levocarnitine	Mar 9, 2011 At notifier's request, FDA ceased to evaluate the notice	4	416
GRN-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]	Mar 26, 2012 FDA has no questions	30	420
GRN-444	Milk protein concentrate and milk protein isolate	Mar 18, 2013 At notifier's request, FDA ceased to evaluate the notice	19	450

¹ Posted at www.accessdata.fda.gov/scripts/cfn/fcnNavigation.cfm?rpt=grasListing.

² See file labelled "chemicals-in-food-FOIA-1.pdf"

³ See file labelled "chemicals-in-food-FOIA-59.pdf"

⁴ See file labelled "chemicals-in-food-FOIA-340.pdf"



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 records¹ 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

¹ "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.’”

² 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).³

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

³ 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.

Thank you for your prompt attention to this request.

Sincerely,



Tom Neltner, Senior Attorney
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Appendix A: Generally Recognized as Safe (GRAS) Notices and Agency Actions

APPENDIX A
GENERALLY RECOGNIZED AS SAFE (GRAS) NOTICES AND AGENCY ACTIONS¹

GRN #	Title
1	Soy isoflavone extract
35	Hempseed oil
36	Chromium picolinate; <i>Ginkgo biloba</i> leaf extract; and Ginseng extract
37	Whey protein isolate and dairy product solids
59	Hydrogenated starch hydrolysate
66	Milk thistle extract
150	Glucosamine hydrochloride prepared from chitin obtained from <i>Aspergillus niger</i>
224	trans-Resveratrol
225	Catechins from green tea extract
257	<i>gamma</i> -Amino butyric acid
262	Sweet lupin protein
263	Sweet lupin fiber
264	Sweet lupin flour
295	Aqueous extract of <i>Emblica officinalis</i>
322	Aqueous extract of <i>Emblica officinalis</i>
324	Heat-killed <i>Lactobacillus plantarum</i>
340	Theobromine
362	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]
444	Milk protein concentrate and milk protein isolate
¹ 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, which appears to read "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 5

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

Enclosure

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.

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

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.

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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



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.

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

Enclosure



December 7, 1999

Slavik Dushenkov, Ph.D.
Consolidated Growers and Processors, Inc.
P.O. Box 2228
Monterey, CA 93942-2228

Re: GRAS Notice (GRN) No. 000035

Dear Dr. Dushenkov:

The Food and Drug Administration (FDA) has received the notice, dated November 16, 1999, that you 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)). FDA received this notice on November 17, 1999 and designated it as GRN No. 000035.

The subject of your notice is hempseed oil. The notice informs FDA of the view of Consolidated Growers and Processors, Inc. that hempseed oil is GRAS, through experience based on common use in food, for use as a flavoring agent, adjuvant solvent, vehicle, stabilizer, thickener, emulsifier, or texturizer in food at the minimum amount required to produce the intended technical effect.

In accordance with proposed § 170.36(f), a copy of the information in your notice that conforms to the information described in proposed § 170.36(c)(1) is available for public review and copying on the Office of Premarket Approval's homepage on the World Wide Web. If you have any questions about your notice, please feel free to contact me at (202) 418-3079.

Sincerely yours,

Paulette M. Gaynor, Ph.D.
Division of Petition Control, HFS-215
Office of Premarket Approval
Center for Food Safety
and Applied Nutrition

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DEPARTMENT OF HEALTH AND HUMAN SERVICES
Memorandum of Telephone Conversation

Dates: January 7, 2000

Between: Paulette Gaynor, Ph.D. (HFS-215)

and

Slavik Dushenkov, Ph.D. (732)-932-8165 extension 110

Subject: GRN 000035 - References cited by the notifier

On January 7, 2000 I telephoned Dr. Dushenkov to request that he send us a copy of four of the references in this GRN. [I telephoned him at the number that he provided in a message that he left for Dr. Linda Kahl.] I explained to Dr. Dushenkov that these references were for clarification and then informed him which of the four references that we would like him to send us a copy. Three of the references are listed on the reference list on the notifier's page 12 and the fourth is cited in the text discussion on the notifier's page 8.

The three references as listed on the reference list on the notifier's page 12 are:

- (1) Executive Order 10480, (1953). The provisions of Executive Order 10480 of Aug. 14, 1953. 18 FR 4939, 3 CFR, 1949-1953 Comp. 1999. <http://www.nara.gov/fedreg/eos/e10480.html>.
- (2) Letter (1995). Department of Agriculture Office of the Secretary, Mr. Dan Glickman, June 19, 1995, re: Ms. Debby Moore president, Kansas Environmentalists for Commerce in Hemp.
- (3) Letter (1999). Office of Consumer Education, FDA, CFSAN, Lyn Goosens, MPH, RD, September 9, 1999, re: Hempseed Oil Safety as Food.

The reference as cited in the text discussion on the notifier's page 8 is Hicks (1986):

Dr. Dushenkov told me he would send a copy of each of these references. OPA received these copies on January 19, 2000.

Paulette M. Gaynor, Ph.D.

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(b) (5)								



DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration
Memorandum of Telephone Conversation

Dates: April 7, June 9, June 12, and August 18, 2000

Between: Linda Kahl, Ph.D. (HFS-206)
and
Slavik Dushenkov, Ph.D., Consolidated Growers and Processors, Inc. (CGP)
(917)748-6071

Subject: GRAS Notice GRN 000035 (Hempseed Oil)

On April 7, 2000, Dr. Dushenkov called me to ask about the status of FDA's review of GRN 000035. I informed Dr. Dushenkov that it was my view that the information presented in GRN 000035 appeared to demonstrate limited food use of hempseed oil before January 1, 1958, but did not demonstrate a broad use that would meet the criteria for common use in food as defined in 21 CFR 170.3(f) (substantial history of consumption of a substance for food use by a significant number of consumers). For example, some of the cited references addressed the use of hemp seed rather than hempseed oil. In addition, the references that addressed the use of hempseed oil appeared to limit the evidence of use of hempseed oil to a single geographic location - i.e., the Ukraine. Given these facts, I informed Dr. Dushenkov that I would recommend that FDA respond that GRN 000035 does not provide a basis that the intended use of hempseed oil is GRAS through experience based on common use in food.

Dr. Dushenkov expressed his view that the intended use of hempseed oil is safe. He asked about other options to engage with FDA about the use of hempseed oil in food. I described the processes for a food additive petition and for a GRAS notice based on scientific procedures. I also described general components of a safety evaluation for an oil that would be used in food. These included the composition of the oil and a comparison of the components of the oil to the components of commonly consumed food; constituents of the source; contaminants that could be introduced by the method of manufacture; and specifications, particularly for any contaminating lead. I also stated that it was my understanding that products derived from hemp could be subject to regulation by the Drug Enforcement Administration and suggested that he investigate this possibility and address it in any amendments to GRN 000035 or in any new submission to FDA.

Dr. Dushenkov stated that he would like to determine whether there are additional references to support his view that the use of hempseed oil had been widespread before 1958 before deciding how to proceed. He indicated that he would get back to me in about one month.

On June 9, 2000, I telephoned Dr. Dushenkov because two months had elapsed since our telephone conversation on April 7. I asked Dr. Dushenkov what he had decided to do regarding GRN 000035. Dr. Dushenkov replied that he had not found additional support for his view that the GRAS status of hempseed oil could be established through common use in food but maintained his view that the use of hempseed oil is safe. Dr. Dushenkov then informed me that he intended to write to FDA and request that we cease to evaluate GRN 000035. I requested that he send such a letter by June 16, 2000, and suggested that he transmit the letter by telefax, with a hard copy to follow.

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On June 12, 2000, I received a telephone message from Dr. Dushenkov. In his message, Dr. Dushenkov informed me that CGP had decided to take no action at this time and would await a response from FDA.

On August 16, 2000, I received a telephone call from an individual who asked whether the intended use of hempseed oil "in food" included meat products. As a result of that call, I realized that OPA should have contacted Dr. Dushenkov early in the review process to determine the answer to that question, and send a copy of GRN 000035 to the Food Safety and Inspection Service (FSIS) of the U.S. Department of Agriculture if the intended use did indeed include use in meat or poultry products. I contacted Dr. Robert Post of FSIS ((202)205-0279) and explained that my recommendation that OPA conclude that GRN 000035 does not provide a basis for a GRAS determination was with Dr. Rulis, but I did not know whether he would accept my recommendation. Dr. Post and I agreed that, under the circumstances, it would be appropriate for OPA to refrain from sending the notice for FSIS' review, even if the notifier intended that hempseed oil be used in meat or poultry products.

On August 18, 2000, I received a second phone call from the individual who was interested in using hempseed oil in meat products. This individual was trying to ascertain whether he should proceed on his own to submit a GRAS notice for the use of hempseed oil in meat products. I suggested that he discuss the nature of his request with the notifier. Given that I had referred the this individual to Dr. Dushenkov, I called Dr. Dushenkov and asked whether he intended to use hempseed oil in meat or poultry products. Dr. Dushenkov replied that he considered the use "in food" to include meat or poultry products. I explained that I had spoken with Dr. Post and would not send the notice for FSIS' review at this time. I stated that I would send the notice to FSIS if Dr. Rulis disagreed with my recommendation to conclude that the notice does not provide a basis for a GRAS determination. I also stated that, if Dr. Rulis disagreed with my current recommendation, I would not delay our response if FSIS required more time to evaluate the notice than we needed to re-draft our letter responding to the notice.

~~Linda S. Kahl~~
Linda S. Kahl
9/1/00

000054



DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service
Food and Drug Administration

Memorandum

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Date August 25, 2000

From Consumer Safety Officer and GRAS Team Coordinator

Subject GRN 000035

To Administrative File, GRN 000035

This memorandum summarizes the notice dated November 16, 1999, that Consolidated Growers and Processors, Inc. (CGP) 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)). This memorandum also includes regulatory policy recommendations and, thus, is signed by the GRAS Team Coordinator in addition to the Consumer Safety Officer who chaired this notice.

The Office of Premarket Approval (OPA) received the notice on November 17, 1999, and designated it as GRN 000035. The subject of the notice is hempseed oil. The notice informs FDA of the view of CGP that hempseed oil is GRAS, through experience based on common use in food, for use as a flavoring agent, adjuvant solvent, vehicle, stabilizer, thickener, emulsifier, or texturizer in food at the minimum amount required to produce the intended technical effect.

Data and information that CGP presents to support its GRAS determination

We summarize below the information that CGP presents to support its position that the use of hempseed oil in food is GRAS through experience based on common use in food.

Item	Reference	Description
1	Markevich, 1860	Two examples of hempseed oil used in Ukrainian recipes (i.e., Borshch and Kutia)
2	Plotnikov, 1931	Hempseed oil poured over buckwheat porridge was eaten by lumberjacks in the Ukraine in 1931
3	Ivanoff, 1938	Hempseed oil was used in the fish-canning industry and in the confectionery industry during the 1930's in the Ukraine
4	Goloborod'ko 1995	Hempseed oil was used in the Ukraine for food and technical purposes.
5	Hicks, 1896 ¹	1895 Yearbook of the United States Department of Agriculture (USDA). This reference states, in part, that hempseed oil sometimes was used as an illuminant and rarely was used for food.

¹In response to a telephone request from Dr. Paulette Gaynor on January 7, 2000, CGP provided a copy of this reference. Although the notice describes the date of this reference as "1986," the copy that CGP provided indicates that this reference dates from 1896 rather than 1986.

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Item	Reference	Description
6	Dewey, 1913	According to ancient Chinese writings, hemp seeds were used for a variety of uses including fiber, oil and as a medicine
7	Clarke and Gu, 1998	Hmong people of the China/Vietnam border region preserved the ancient tradition of hemp use (e.g., use of hemp seed)
8	Li, 1974	Hemp seeds remained a staple of the Chinese diet through the 10 th century
9	Bretschneider, 1893	Hemp ("ma") seeds used for food in China
10	Birrenbach (Internet)	Hemp seed was cooked with barley and other grains and eaten.
11	Executive Order (EO) 10480	EO 10480 defines "food" for the purpose of the Executive Order. That definition of food includes hemp.
12	Letter dated June 19, 1995, from the Secretary of USDA	References section 901(e) of an Executive order (i.e., Executive Order 12919).
13	Electronic mail message from the Consumer Education Staff	Hempseed oil is an eatable oil

Evaluation of the data and information in GRN 000035

Under section 201(s) of the Federal Food, Drug, and Cosmetic Act (the FFDCA), a substance that is added to food is not subject to the requirement for premarket approval as a food additive if its safety is generally recognized, among qualified experts, through experience based on common use in food. Under 21 CFR 170.3(f), "common use in food" requires a substantial history of consumption for food use by a significant number of consumers. Thus, the fact that something may be used as a food does not, in itself, demonstrate that such use is safe, unless that use is sufficiently broad to demonstrate to qualified experts that the use in food demonstrates safety. Accordingly, we evaluated CGP's submission in this context.

We evaluated Items 1 through 4 (in the table above) under the standard set forth in 21 CFR 170.3(f). In our view, the limited use of hempseed oil that these references describe does not constitute evidence of a substantial history of consumption for food use that is required under 21 CFR 170.3(f) to demonstrate safety.

We also evaluated Item 5 under the standard set forth in 21 CFR 170.3(f). In our view, the referenced statement that hempseed oil rarely was used for food undermines CGP's position that hempseed oil was commonly used in food prior to 1958.

Under section 201(s) of the FFDCA, a GRAS substance must be generally recognized as safe

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"under the conditions of its intended use." Because Items 6 through 10 describe the use of hemp seeds rather than hempseed oil, we did not consider these references to be relevant to the intended use that CGP describes in its notice.

We obtained a copy of Executive Order 12919 (see attachment), which supersedes Executive Order 10480. Executive Order 12919 delegates authorities and addresses national defense industrial resource policies and programs under the Defense Production Act of 1950. Executive Order 12919 defines "food resources" for the purpose of the Executive Order as a variety of agricultural commodities, including wool, tobacco, mohair and other materials. Given this context, it is our view that this Executive Order does not provide evidence of experience based on common use in food within the meaning of section 201(s) of the FFDCA.

The electronic mail message from the Consumer Education Staff in FDA's Center for Food Safety and Applied Nutrition answered a question from a consumer. That question was whether hempseed oil has been approved by FDA for use as a food or as a food additive. The response from the Consumer Education Staff does not address the question, raised by CGP's notice, whether there was a substantial history of consumption for food use by a significant number of consumers prior to 1958. Moreover, under 21 CFR 10.85(k), the opinion expressed in that electronic mail message that hempseed oil is an edible oil is an informal opinion that represents the best judgment of the Consumer Education Staff at that time; that electronic mail message does not necessarily represent the formal position of FDA and does not bind or otherwise obligate or commit the agency to the views expressed.

On April 7, 2000, Dr. Dushenkov telephoned Dr. Linda Kahl to ask about the status of FDA's review of GRN 000035. As discussed more thoroughly in a memorandum of this telephone conversation, and of subsequent telephone conversations on June 9, 2000, and June 12, 2000, Dr. Kahl informed Dr. Dushenkov that it was her view that the information presented in GRN 000035 appeared to demonstrate limited food use of hempseed oil before January 1, 1958, but did not demonstrate a broad use that would meet the criteria for common use in food as defined in 21 CFR 170.3(f).

Conclusions

CGP's notice describes some use of hempseed oil within one foreign country. CGP's notice also cites a reference that hempseed oil rarely was used for food; describes uses of the related substance, hemp seed; references an Executive Order that includes hemp and several other inedible products in its definition of food resources for the purposes of that Executive Order; and cites an electronic mail message of an FDA employee in response to a question from a consumer about whether hempseed oil has been approved by FDA.

We have evaluated the information that CGP discusses in its GRAS notice as well as other data and information that are available to the agency. In our view, the information that CGP discusses in its notice does not provide evidence of a substantial history of consumption for food use by a significant number of consumers. Given that CGP based its GRAS determination on common use in food, rather than through scientific procedures, we did not request a scientific evaluation of whether there are any issues that would contradict the view of CGP is safe under the conditions of its intended use.


~~Paulette M. Gaynor~~
Paulette M. Gaynor, Ph.D.

~~Linda S. Kahl~~
Linda S. Kahl, Ph.D.

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Attachment: Executive Order 12919

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(2) Provide for the central coordination of the plans and programs incident to authorities and functions delegated under this order, and provide guidance and procedures approved by the Assistant to the President for National Security Affairs to the Federal departments and agencies under this order;

(3) Establish procedures, in consultation with Federal departments and agencies assigned functions under this order, to resolve in a timely and effective manner conflicts and issues that may arise in implementing the authorities and functions delegated under this order; and

(4) Report to the President periodically concerning all program activities conducted pursuant to this order.

(c) The head of every Federal department and agency assigned functions under this order shall ensure that the performance of these functions is consistent with National Security Council policy and guidelines.

PART II—PRIORITIES AND ALLOCATIONS

Sec. 201. Delegations of Priorities and Allocations. (a) The authority of the President conferred by section 101 of the Act to require acceptance and priority performance of contracts or orders (other than contracts of employment) to promote the national defense over performance of any other contracts or orders, and to allocate materials, services, and facilities as deemed necessary or appropriate to promote the national defense, is delegated to the following agency heads:

(1) The Secretary of Agriculture with respect to food resources, food resource facilities, and the domestic distribution of farm equipment and commercial fertilizer;

(2) The Secretary of Energy with respect to all forms of energy;

(3) The Secretary of Health and Human Services with respect to health resources;

(4) The Secretary of Transportation with respect to all forms of civil transportation;

(5) The Secretary of Defense with respect to water resources; and

(6) The Secretary of Commerce for all other materials, services, and facilities, including construction materials.

(b) The Secretary of Commerce, in consultation with the heads of those departments and agencies specified in subsection 201(a) of this order, shall administer the Defense Priorities and Allocations System ("DPAS") regulations that will be used to implement the authority of the President conferred by section 101 of the Act as delegated to the Secretary of Commerce in subsection 201(a)(6) of this order. The Secretary of Commerce will redelegate to the Secretary of Defense, and the heads of other departments and agencies as appropriate, authority for the priority rating of contracts and orders for all materials, services, and facilities needed in support of programs approved under section 202 of this order. The Secretary of Commerce shall act as appropriate upon Special Priorities Assistance requests in a time frame consistent with the urgency of the need at hand.

(c) The Director, FEMA, shall attempt to resolve issues or disagreements on priorities or allocations between Federal departments or agencies in a time frame consistent with the urgency of the issue at hand and, if not resolved, such issues will be referred to the Assistant to the President for National Security Affairs for final determination.

(d) The head of each Federal department or agency assigned functions under subsection 201(a) of this order, when necessary, shall make the finding required under subsection 101(b) of the Act. This finding shall be submitted for the President's approval through the Assistant to the President for National Security Affairs. Upon such approval the head of the Federal depart-

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ment or agency that made the finding may use the authority of subsection 101(a) of the Act to control the general distribution of any material (including applicable services) in the civilian market.

(e) The Assistant to the President for National Security Affairs is hereby delegated the authority under subsection 101(c)(3) of the Act, and will be assisted by the Director, FEMA, in ensuring the coordinated administration of the Act.

Sec. 202. Determinations. The authority delegated by section 201 of this order may be used only to support programs that have been determined in writing as necessary or appropriate to promote the national defense:

(a) By the Secretary of Defense with respect to military production and construction, military assistance to foreign nations, stockpiling, outer space, and directly related activities;

(b) By the Secretary of Energy with respect to energy production and construction, distribution and use, and directly related activities; and

(c) By the Director, FEMA, with respect to essential civilian needs supporting national defense, including civil defense and continuity of government and directly related activities.

Sec. 203. Maximizing Domestic Energy Supplies. The authority of the President to perform the functions provided by subsection 101(c) of the Act is delegated to the Secretary of Commerce, who shall redelegate to the Secretary of Energy the authority to make the findings described in subsection 101(c)(2)(A) that the materials (including equipment), services, and facilities are critical and essential. The Secretary of Commerce shall make the finding described in subsection 101(c)(2)(A) of the Act that the materials (including equipment), services, or facilities are scarce, and the finding described in subsection 101(c)(2)(B) that it is necessary to use the authority provided by subsection 101(c)(1).

Sec. 204. Chemical and Biological Warfare. The authority of the President conferred by subsection 104(b) of the Act is delegated to the Secretary of Defense. This authority may not be further delegated by the Secretary.

PART III—EXPANSION OF PRODUCTIVE CAPACITY AND SUPPLY

Sec. 301. (a) Financing Institution Guarantees. To expedite or expand production and deliveries or services under government contracts for the procurement of industrial resources or critical technology items essential to the national defense, the head of each Federal department or agency engaged in procurement for the national defense (referred to as "agency head" in this part) and the President and Chairman of the Export-Import Bank of the United States (in cases involving capacity expansion, technological development, or production in foreign countries) are authorized to guarantee in whole or in part any public or private financing institution, subject to provisions of section 301 of the Act. Guarantees shall be made in consultation with the Department of the Treasury as to the terms and conditions thereof. The Director of the Office of Management and Budget ("OMB") shall be informed when such guarantees are to be made.

(b) *Direct Loan Guarantees.* To expedite or expand production and deliveries or services under government contracts for the procurement of industrial resources or critical technology items essential to the national defense, each agency head is authorized to make direct loan guarantees from funds appropriated to their agency for Title III.

(c) *Fiscal Agent.* Each Federal Reserve Bank is designated and authorized to act, on behalf of any guaranteeing agency, as fiscal agent in the making of guarantee contracts and in otherwise carrying out the purposes of section 301 of the Act.

(d) *Regulations.* The Board of Governors of the Federal Reserve System is authorized, after consultation with heads of guaranteeing departments and agencies, the Secretary of the Treasury, and the Director, OMB, to

prescribe regulations governing procedures, forms, rates of interest, and fees for such guarantee contracts.

Sec. 302. Loans. (a) To expedite production and deliveries or services to aid in carrying out government contracts for the procurement of industrial resources or a critical technology item for the national defense, an agency head is authorized, subject to the provisions of section 302 of the Act, to submit to the Secretary of the Treasury or the President and Chairman of the Export-Import Bank of the United States (in cases involving capacity expansion, technological development, or production in foreign countries) applications for loans.

(b) To expedite or expand production and deliveries or services under government contracts for the procurement of industrial resources or critical technology items essential to the national defense, each agency head may make direct loans from funds appropriated to their agency for Title III.

(c) After receiving a loan application and determining that financial assistance is not otherwise available on reasonable terms, the Secretary of the Treasury or the President and Chairman of the Export-Import Bank of the United States (in cases involving capacity expansion, technological development, or production in foreign countries) may make loans, subject to provisions of section 302 of the Act.

Sec. 303. Purchase Commitments. (a) In order to carry out the objectives of the Act, and subject to the provisions of section 303 thereof, an agency head is authorized to make provision for purchases of, or commitments to purchase, an industrial resource or a critical technology item for government use or resale.

(b) Materials acquired under section 303 of the Act that exceed the needs of the programs under the Act may be transferred to the National Defense Stockpile, if such transfer is determined by the Secretary of Defense as the National Defense Stockpile Manager to be in the public interest.

Sec. 304. Subsidy Payments. In order to ensure the supply of raw or non-processed materials from high-cost sources, an agency head is authorized to make subsidy payments, after consultation with the Secretary of the Treasury and the Director, OMB, and subject to the provisions of section 303(c) of the Act.

Sec. 305. Determinations and Findings. When carrying out the authorities in sections 301 through 303 of this order, an agency head is authorized to make the required determinations, judgments, statements, certifications, and findings, in consultation with the Secretary of Defense, Secretary of Energy or Director, FEMA, as appropriate. The agency head shall provide a copy of the determination, judgment, statement, certification, or finding to the Director, OMB, to the Director, FEMA, and, when appropriate, to the Secretary of the Treasury.

Sec. 306. Strategic and Critical Materials. (a) The Secretary of the Interior, in consultation with the Secretary of Defense as the National Defense Stockpile Manager and subject to the provisions of section 303 of the Act, is authorized to encourage the exploration, development, and mining of critical and strategic materials and other materials.

(b) An agency head is authorized, pursuant to section 303(g) of the Act, to make provision for the development of substitutes for strategic and critical materials, critical components, critical technology items, and other industrial resources to aid the national defense.

(c) An agency head is authorized, pursuant to section 303(a)(1)(B) of the Act, to make provisions to encourage the exploration, development, and mining of critical and strategic materials and other materials.

Sec. 307. Government-owned Equipment. An agency head is authorized, pursuant to section 303(e) of the Act, to install additional equipment, facilities, processes, or improvements to facilities owned by the government and to install government-owned equipment in industrial facilities owned by private persons.

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Sec. 308. Identification of Shortfalls. Except during periods of national emergency or after a Presidential determination in accordance with sections 301(e)(1)(D)(ii), 302(c)(4)(B), or 303(a)(7)(B) of the Act, no guarantee, loan or other action pursuant to sections 301, 302, and 303 of the Act to correct an industrial shortfall shall be taken unless the shortfall has been identified in the Budget of the United States or amendments thereto.

Sec. 309. Defense Production Act Fund Manager. The Secretary of Defense is designated the Defense Production Act Fund Manager, in accordance with section 304(f) of the Act, and shall carry out the duties specified in that section, in consultation with the agency heads having approved Title III projects and appropriated Title III funds.

Sec. 310. Critical Items List. (a) Pursuant to section 107(b)(1)(A) of the Act, the Secretary of Defense shall identify critical components and critical technology items for each item on the Critical Items List of the Commanders-in-Chief of the Unified and Specified Commands and other items within the inventory of weapon systems and defense equipment.

(b) Each agency head shall take appropriate action to ensure that critical components or critical technology items are available from reliable sources when needed to meet defense requirements during peacetime, graduated mobilization, and national emergency. "Appropriate action" may include restricting contract solicitations to reliable sources, restricting contract solicitations to domestic sources (pursuant to statutory authority), stockpiling critical components, and developing substitutes for critical components or critical technology items.

Sec. 311. Strengthening Domestic Capability. An agency head, in accordance with section 107(a) of the Act, may utilize the authority of Title III of the Act or any other provision of law, in consultation with the Secretary of Defense, to provide appropriate incentives to develop, maintain, modernize, and expand the productive capacities of domestic sources for critical components, critical technology items, and industrial resources essential for the execution of the national security strategy of the United States.

Sec. 312. Modernization of Equipment. An agency head, in accordance with section 108(b) of the Act, may utilize the authority of Title III of the Act to guarantee the purchase or lease of advance manufacturing equipment and any related services with respect to any such equipment for purposes of the Act.

PART IV—IMPACT OF OFFSETS

Sec. 401. Offsets. (a) The responsibilities and authority conferred upon the President by section 309 of the Act with respect to offsets are delegated to the Secretary of Commerce, who shall function as the President's Executive Agent for carrying out this authority.

(b) The Secretary of Commerce shall prepare the annual report required by section 309(a) of the Act in consultation with the Secretaries of Defense, Treasury, Labor, State, the United States Trade Representative, the Arms Control and Disarmament Agency, the Director of Central Intelligence, and the heads of other departments and agencies as required. The heads of Federal departments and agencies shall provide the Secretary of Commerce with such information as may be necessary for the effective performance of this function.

(c) The offset report shall be subject to the normal interagency clearance process conducted by the Director, OMB, prior to the report's submission by the President to Congress.

PART V—VOLUNTARY AGREEMENTS AND ADVISORY COMMITTEES

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Sec. 501. Appointments. The authority of the President under sections 708(c) and (d) of the Act is delegated to the heads of each Federal department or agency, except that, insofar as that authority relates to section 101 of the Act, it is delegated only to the heads of each Federal department or agency assigned functions under section 201(a) of this order. The authority

delegated under this section shall be exercised pursuant to the provisions of section 708 of the Act, and copies and the status of the use of such delegations shall be furnished to the Director, FEMA.

Sec. 502. Advisory Committees. The authority of the President under section 708(d) of the Act and delegated in section 501 of this order (relating to establishment of advisory committees) shall be exercised only after consultation with, and in accordance with, guidelines and procedures established by the Administrator of General Services.

PART VI—EMPLOYMENT OF PERSONNEL

Sec. 601. National Defense Executive Reserve. (a) In accordance with section 710(e) of the Act, there is established in the Executive Branch a National Defense Executive Reserve ("NDER") composed of persons of recognized expertise from various segments of the private sector and from government (except full-time federal employees) for training for employment in executive positions in the Federal Government in the event of an emergency that requires such employment.

(b) The head of any department or agency may establish a unit of the NDER in the department or agency and train members of that unit.

(c) The head of each department or agency with an NDER unit is authorized to exercise the President's authority to employ civilian personnel in accordance with section 703(a) of the Act when activating all or a part of its NDER unit. The exercise of this authority shall be subject to the provisions of subsections 601(d) and (e) of this order and shall not be redelegated.

(d) The head of a department or agency may activate an NDER unit, in whole or in part, upon the written determination that an emergency affecting the national security or defense preparedness of the United States exists and that the activation of the unit is necessary to carry out the emergency program functions of the department or agency.

(e) At least 72 hours prior to activating the NDER unit, the head of the department or agency shall notify, in writing, the Assistant to the President for National Security Affairs of the impending activation and provide a copy of the determination required under subsection 601(d) of this order.

(f) The Director, FEMA, shall coordinate the NDER program activities of departments and agencies in establishing units of the Reserve; provide for appropriate guidance for recruitment, training, and activation; and issue necessary rules and guidance in connection with the program.

(g) This order suspends any delegated authority, regulation, or other requirement or condition with respect to the activation of any NDER unit, in whole or in part, or appointment of any NDER member that is inconsistent with the authorities delegated herein, provided that the aforesaid suspension applies only as long as sections 703(a) and 710(e) of the Act are in effect.

Sec. 602. Consultants. The head of each department or agency assigned functions under this order is delegated authority under sections 710(b) and (c) of the Act to employ persons of outstanding experience and ability without compensation and to employ experts, consultants, or organizations. The authority delegated by this section shall not be redelegated.

PART VII—LABOR SUPPLY

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Sec. 701. Secretary of Labor. The Secretary of Labor, identified in this section as the Secretary, shall:

(a) Collect, analyze, and maintain data needed to make a continuing appraisal of the nation's labor requirements and the supply of workers for purposes of national defense. All agencies of the government shall cooperate with the Secretary in furnishing information necessary for this purpose, to the extent permitted by law;

(b) In response to requests from the head of a Federal department or agency engaged in the procurement for national defense, consult with and advise that department or agency with respect to (1) the effect of contemplated

actions on labor supply and utilization, (2) the relation of labor supply to materials and facilities requirements, and (3) such other matters as will assist in making the exercise of priority and allocations functions consistent with effective utilization and distribution of labor;

(c) Formulate plans, programs, and policies for meeting defense and essential civilian labor requirements;

(d) Project skill shortages to facilitate meeting defense and essential civilian needs and establish training programs;

(e) Determine the occupations and skills critical to meeting the labor requirements of defense and essential civilian activities and, with the assistance of the Secretary of Defense, the Director of Selective Service, and such other persons as the Director, FEMA, may designate, develop policies regulating the induction and deferment of personnel for the armed services, except for civilian personnel in the reserves; and

(f) Administer an effective labor-management relations policy to support the activities and programs under this order with the cooperation of other Federal agencies, including the National Labor Relations Board and the Federal Mediation and Conciliation Service.

PART VIII—DEFENSE INDUSTRIAL BASE INFORMATION AND REPORTS

Sec. 801. Foreign Acquisition of Companies. The Secretary of the Treasury, in cooperation with the Department of State, the Department of Defense, the Department of Commerce, the Department of Energy, the Department of Agriculture, the Attorney General, and the Director of Central Intelligence, shall complete and furnish a report to the President and then to Congress in accordance with the requirements of section 721(k) of the Act concerning foreign efforts to acquire United States companies involved in research, development, or production of critical technologies and industrial espionage activities directed by foreign governments against private U.S. companies.

Sec. 802. Defense Industrial Base Information System. (a) The Secretary of Defense and the heads of other appropriate Federal departments and agencies, as determined by the Secretary of Defense, shall establish an information system on the domestic defense industrial base in accordance with the requirements of section 722 of the Act.

(b) In establishing the information system required by subsection (a) of this order, the Secretary of Defense, the Secretary of Commerce, and the heads of other appropriate Federal departments and agencies, as determined by the Secretary of Defense in consultation with the Secretary of Commerce, shall consult with each other for the purposes of performing the duties listed in section 722(d)(1) of the Act.

(c) The Secretary of Defense shall convene a task force consisting of the Secretary of Commerce and the Secretary of each military department and the heads of other appropriate Federal departments and agencies, as determined by the Secretary of Defense in consultation with the Secretary of Commerce, to carry out the duties under section 722(d)(2) of the Act.

(d) The Secretary of Defense shall report to Congress on a strategic plan for developing a cost-effective, comprehensive information system capable of identifying on a timely, ongoing basis vulnerability in critical components and critical technology items. The plans shall include an assessment of the performance and cost-effectiveness of procedures specified in section 722(b) of the Act.

(e) The Secretary of Commerce, acting through the Bureau of the Census, shall consult with the Secretary of Defense and the Director, FEMA, to improve the usefulness of information derived from the Census of Manufacturers in carrying out section 722 of the Act.

(f) The Secretary of Defense shall perform an analysis of the production base for not more than two major weapons systems of each military depart-

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ment in establishing the information system under section 722 of the Act. Each analysis shall identify the critical components of each system.

(g) The Secretary of Defense, in consultation with the Secretary of Commerce, and the heads of other Federal departments and agencies as appropriate, shall issue a biennial report on critical components and technology in accordance with section 722(e) of the Act.

PART IX—GENERAL PROVISIONS

Sec. 901. Definitions. In addition to the definitions in section 702 of the Act, the following definitions apply throughout this order:

(a) "Civil transportation" includes movement of persons and property by all modes of transportation in interstate, intrastate, or foreign commerce within the United States, its territories and possessions, and the District of Columbia, and, without limitation, related public storage and warehousing, ports, services, equipment and facilities, such as transportation carrier shop and repair facilities. However, "civil transportation" shall not include transportation owned or controlled by the Department of Defense, use of petroleum and gas pipelines, and coal slurry pipelines used only to supply energy production facilities directly. As applied herein, "civil transportation" shall include direction, control, and coordination of civil transportation capacity regardless of ownership.

(b) "Energy" means all forms of energy including petroleum, gas (both natural and manufactured), electricity, solid fuels (including all forms of coal, coke, coal chemicals, coal liquification, and coal gasification), and atomic energy, and the production, conservation, use, control, and distribution (including pipelines) of all of these forms of energy.

(c) "Farm equipment" means equipment, machinery, and repair parts manufactured for use on farms in connection with the production or preparation for market use of food resources.

(d) "Fertilizer" means any product or combination of products that contain one or more of the elements—nitrogen, phosphorus, and potassium—for use as a plant nutrient.

(e) "Food resources" means all commodities and products, simple, mixed, or compound, or complements to such commodities or products, that are capable of being ingested by either human beings or animals, irrespective of other uses to which such commodities or products may be put, at all stages of processing from the raw commodity to the products thereof in vendible form for human or animal consumption. "Food resources" also means all starches, sugars, vegetable and animal or marine fats and oils, cotton, tobacco, wool, mohair, hemp, flax fiber, and naval stores, but does not mean any such material after it loses its identity as an agricultural commodity or agricultural product.

(f) "Food resource facilities" means plants, machinery, vehicles (including on-farm), and other facilities required for the production, processing, distribution, and storage (including cold storage) of food resources, livestock and poultry feed and seed, and for the domestic distribution of farm equipment and fertilizer (excluding transportation thereof).

(g) "Functions" include powers, duties, authority, responsibilities, and discretion.

(h) "Head of each Federal department or agency engaged in procurement for the national defense" means the heads of the Departments of Defense, Energy, and Commerce, as well as those departments and agencies listed in Executive Order No. 10789.

(i) "Heads of other appropriate Federal departments and agencies" as used in part VIII of this order means the heads of such other Federal agencies and departments that acquire information or need information with respect to making any determination to exercise any authority under the Act.

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(j) "Health resources" means materials, facilities, health supplies, and equipment (including pharmaceutical, blood collecting and dispensing supplies, biological, surgical textiles, and emergency surgical instruments and supplies) required to prevent the impairment of, improve, or restore the physical and mental health conditions of the population.

(k) "Metals and minerals" means all raw materials of mineral origin (excluding energy) including their refining, smelting, or processing, but excluding their fabrication.

(l) "Strategic and Critical Materials" means materials (including energy) that (1) would be needed to supply the military, industrial, and essential civilian needs of the United States during a national security emergency, and (2) are not found or produced in the United States in sufficient quantities to meet such need and are vulnerable to the termination or reduction of the availability of the material.

(m) "Water resources" means all usable water, from all sources, within the jurisdiction of the United States, which can be managed, controlled, and allocated to meet emergency requirements.

Sec. 902. General. (a) Except as otherwise provided in subsection 902(c) of this order, the authorities vested in the President by title VII of the Act may be exercised and performed by the head of each department and agency in carrying out the delegated authorities under the Act and this order.

(b) The authorities which may be exercised and performed pursuant to subsection 902(a) of this order shall include (1) the power to redelegate authorities, and to authorize the successive redelegation of authorities, to departments and agencies, officers, and employees of the government, and (2) the power of subpoena with respect to authorities delegated in parts II, III, and IV of this order, provided that the subpoena power shall be utilized only after the scope and purpose of the investigation, inspection, or inquiry to which the subpoena relates have been defined either by the appropriate officer identified in subsection 902(a) of this order or by such other person or persons as the officer shall designate.

(c) Excluded from the authorities delegated by subsection 902(a) of this order are authorities delegated by parts V, VI, and VIII of this order and the authority with respect to fixing compensation under section 703(a) of the Act.

Sec. 903. Authority. All previously issued orders, regulations, rulings, certificates, directives, and other actions relating to any function affected by this order shall remain in effect except as they are inconsistent with this order or are subsequently amended or revoked under proper authority. Nothing in this order shall affect the validity or force of anything done under previous delegations or other assignment of authority under the Act.

Sec. 904. Effect on other Orders. (a) The following are superseded or revoked:

- (1) Section 3, Executive Order No. 8248 of September 8, 1939, (4 FR 3864).
- (2) Executive Order No. 10222 of March 8, 1951 (16 FR 2247).
- (3) Executive Order No. 10480 of August 14, 1953 (18 FR 4939).
- (4) Executive Order No. 10647 of November 28, 1955 (20 FR 8769).
- (5) Executive Order No. 11179 of September 22, 1964 (29 FR 13239).
- (6) Executive Order No. 11355 of May 26, 1967 (32 FR 7803).
- (7) Sections 7 and 8, Executive Order No. 11912 of April 13, 1976 (41 FR 15825, 15826-27).
- (8) Section 3, Executive Order No. 12148 of July 20, 1979 (44 FR 43239, 43241).
- (9) Executive Order No. 12521 of June 24, 1985 (50 FR 26335).
- (10) Executive Order No. 12649 of August 11, 1988 (53 FR 30639).

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(11) Executive Order No. 12773 of September 26, 1991 (56 FR 49387), except that part of the order that amends section 604 of Executive Order 10480.

(b) Executive Order No. 10789 of November 14, 1958, is amended by deleting "and in view of the existing national emergency declared by Proclamation No. 2914 of December 16, 1950," as it appears in the first sentence.

(c) Executive Order No. 11790, as amended, relating to the Federal Energy Administration Act of 1974, is amended by deleting "Executive Order No. 10480" where it appears in section 4 and substituting this order's number.

(d) Subject to subsection 904(c) of this order, to the extent that any provision of any prior Executive order is inconsistent with the provisions of this order, this order shall control and such prior provision is amended accordingly.

Sec. 905. Judicial Review. This order is not intended to create any right or benefit, substantive or procedural, enforceable at law by a party against the United States, its agencies, its officers, or any person.

William Pinson

THE WHITE HOUSE.

June 3, 1994.

IFR Doc. 94-14027

Filed 6-6-94: 10:45 am

Billing code J195-01-P

000068



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

TO:

~~Dr. Dush...~~

FROM:

~~Dr. G...~~

DATE:

Aug 29, 2000

PHONE:

(202) 418- 3079

FAX:

732-932-6535

FAX:

(202) 418-3131

MESSAGE~~Dr. Dush...~~

Paper copy of the letter was mailed
from our office on August 24, 2000-

~~P. G...~~

000069

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Page 1 of 5

FACSIMILE



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

TO:	Dr. D. A. [unclear]	FROM:	Dr. [unclear]
		DATE:	Aug 29, 2000
		PHONE:	(202) 418- 3079
FAX:	732-932-6535	FAX:	(202) 418-3131

MESSAGE

~~Dr. D. A. [unclear]~~

Paper copy of the letter was mailed
 from our office on August 24, 2000

~~P. [unclear]~~

000069.001

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VANCOL INDUSTRIES, INC.

P.O. BOX 11037, DENVER, CO 80211
1700 EAST 68TH AVE. DENVER, CO 80229
303-289-8655 FAX 303-287-7947

November 19, 1999

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

GRAS Exemption claim for Ginseng used as an ingredient in a non-carbonated beverage or tea.

The information which forms the basis of this claim may be obtained by the FDA from Vancol Industries, Inc at the address on the letterhead of this document.

Notified substance

Ginseng Extract of the dried main and lateral root and root hairs of Panax Ginseng
C.A. Meyer (Fam. Araliaceae)

Proposed condition of use

Extract is proposed as an ingredient in a non-carbonated fruit beverage or tea marketed under the brand name ORA. Distribution will achieve the widest consumer availability possible. Distribution channels will include but not be limited to grocery stores, convenience stores, mass merchandisers and drug store chains.

The extract is an alcohol and water extract produced from the root of Panax Ginseng. 1 gram of Ginseng root yields 5.57 grams of Ginseng extract. The source of the root is the US.

150 milligrams of Ginseng extract is used per 8 oz serving in a container that contains 20 fl. oz. or 2.5 servings. It is reasonable to expect that a consumer may drink 1 or more containers per day.

Ginseng extract is added to ORA beverages because it is widely recognized as a "tonic for invigoration and fortification in times of fatigue and debility, for declining capacity for work and concentration" to quote the German Commission E Monograph. Consumers are strongly interested in consuming products which improve energy and stamina. Ginseng safely meets those needs.

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Scientific basis for GRAS determination

Professor Varro E. Tyler, Ph.D., Sc.D., Dean and Distinguished Professor of Pharmacognosy Emeritus, School of Pharmacy and Pharmacal Sciences, Purdue University has called the German Commission E Monographs "the most accurate information available in the entire world on the safety and efficacy of herbs and phytomedicines."

In 1978, the German *Bundesgesundheitsamt* (Federal Health Agency), now called the Federal Institute for Drugs and Medical Devices, established an expert committee on herbal remedies, composed principally of members proposed by associations of the health professions, to evaluate the safety and efficacy of phytomedicines. This so-called "Commission E" included physicians, pharmacists, pharmacologists, toxicologists, representatives of the pharmaceutical industry, and lay persons.

In its assessments, Commission E actively checks so-called bibliographic data independently. Such data include information obtained from clinical trials, field studies, collections of single cases, scientific literature, including facts published in the standard reference works and expertise of medical associations. If controlled clinical data are lacking, safety and efficacy can still be determined on the basis of information in the literature, the presence of supplemental data supporting clinical results, and significant experimental studies supporting traditional use.

Application of this kind of evaluation process results in the establishment of "reasonable certainty" of the safety and efficacy of the herb being evaluated.

The Monograph for Ginseng root, published January 17, 1991, shows:

Composition:

Ginseng root consists of the dried main and lateral root and root hairs of *Panax ginseng* as well as their preparations. The root contains at least 1.5 percent ginsenosides, calculated as Ginsenoside Rg1.

Dosage:

Unless otherwise prescribed:

Daily dosage: 1-2 grams of root or equivalent preparations.

Side Effects:

None known.

Interactions with Other Drugs

None known

Contraindications:

None known.

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In other words, Ginseng is completely safe. No wonder there are centuries of Ginseng use in everything from teas to brandies and wines to candies.

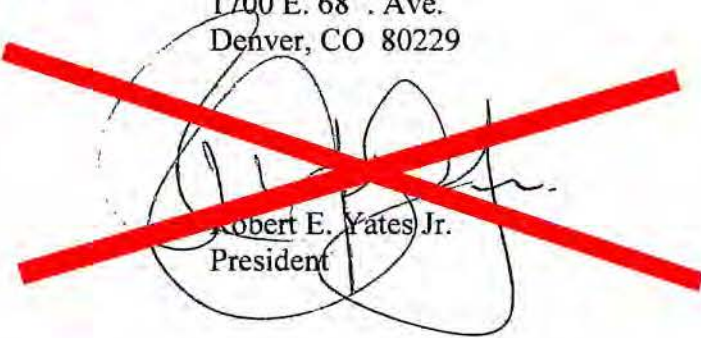
Potential for overconsumption

It is unlikely that a consumer would or could consume enough ORA beverage to create an overdose of Ginseng. Each 20 oz. bottle of ORA contains the equivalent of 67.32 mg. of Ginseng root. A consumer would need to consume nearly 15 bottles to ingest 1 gram equivalent of Ginseng root. Even if the consumer were taking a typical Ginseng supplement of 500 mg. per day, a reasonable level of ORA consumption (1 to 2 bottles) would not create potential for toxicity.

Source of information:

The Complete German Commission E Monographs
Therapeutic Guide to Herbal Medicines
Published by: American Botanical Council
Austin, Texas
1998

Submitted by: Vancol Industries, Inc.
1700 E. 68th Ave.
Denver, CO 80229


Robert E. Yates Jr.
President

1999 NOV 30 P 12:15

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VANCOL INDUSTRIES, INC.

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November 19, 1999

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

GRAS Exemption claim for Ginkgo Biloba Leaf Extract used as an ingredient in a non-carbonated beverage or tea.

The information which forms the basis of this claim may be obtained by the FDA from Vancol Industries, Inc at the address on the letterhead of this document.

Notified substance

Ginkgo Biloba Leaf Extract of the dried leaf of *Ginkgo biloba* L.

Proposed condition of use

Extract is proposed as an ingredient in a non-carbonated fruit beverage or tea marketed under the brand name ORA. Distribution will achieve the widest consumer availability possible. Distribution channels will include but not be limited to grocery stores, convenience stores, mass merchandisers and drug store chains.

The extract is an alcohol and water extract produced from the leaf of Ginkgo Biloba. 1 gram of Ginkgo leaf yields 4.0 grams of Ginkgo Biloba extract.

30 milligrams of Ginkgo Biloba extract is used per 8 oz serving in a container that contains 20 fl. oz. or 2.5 servings. It is reasonable to expect that a consumer may drink 1 or more containers per day.

Ginkgo Biloba extract is added to ORA beverages because it is widely known that Ginkgo Biloba enhances memory and improves cardio vascular function. Consumers have a tremendous interest in consuming products which can help them achieve higher levels of daily performance in their work and play and which can contribute to their overall health and wellbeing. Ginkgo Biloba safely meets those needs.

Scientific basis for GRAS determination

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Professor Varro E. Tyler, Ph.D., Sc.D., Dean and Distinguished Professor of Pharmacognosy Emeritus, School of Pharmacy and Pharmacal Sciences, Purdue University has called the German Commission E Monographs "the most accurate

information available in the entire world on the safety and efficacy of herbs and phytomedicines.”

In 1978, the German *Bundesgesundheitsamt* (Federal Health Agency), now called the Federal Institute for Drugs and Medical Devices, established an expert committee on herbal remedies, composed principally of members proposed by associations of the health professions, to evaluate the safety and efficacy of phytomedicines. This so-called “Commission E” included physicians, pharmacists, pharmacologists, toxicologists, representatives of the pharmaceutical industry, and lay persons.

In its assessments, Commission E actively checks so-called bibliographic data independently. Such data include information obtained from clinical trials, field studies, collections of single cases, scientific literature, including facts published in the standard reference works and expertise of medical associations. If controlled clinical data are lacking, safety and efficacy can still be determined on the basis of information in the literature, the presence of supplemental data supporting clinical results, and significant experimental studies supporting traditional use.

Application of this kind of evaluation process results in the establishment of “reasonable certainty” of the safety and efficacy of the herb being evaluated.

The Monograph for Ginkgo Biloba Leaf Extract, published July 19, 1994, shows:

Composition:

A dry extract (35-67:1) from Ginkgo Biloba L. leaf
(Fam. Ginkgoaceae), extracted with acetone/water.
Drug/extract ratio on average 50:1.

Dosage:

Daily dosage: 120-240 mg native dry extract.

Side Effects:

Very seldom stomach or intestinal upsets, headaches or allergic skin reaction.

Interactions with Other Drugs

None known

Contraindications:

Hypersensitivity to Ginkgo Biloba preparations.

Special Cautions in Use:

None known.

Use During Pregnancy and Lactation

No restrictions known

Overdosage:

None known

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Special Warnings
None.

Effects on Operators of Vehicles and Machinery:
None known

In other words, Ginkgo Biloba is completely safe.

Potential for overconsumption

It is unlikely that a consumer would or could consume enough ORA beverage to create an overdose of Ginkgo Biloba. The two factors which negate potential for overconsumption are the fact that there is no known level at which one can overdose on Ginkgo Biloba. The second factor is the low level of Ginkgo Biloba in each serving of product. Each 20 oz. bottle of ORA contains the equivalent of 18.75 mg. of Ginkgo Biloba leaf equal to 0.375 mg of 50:1 extract used as the standard by Commission E. A consumer would need to consume 320 bottles to ingest a 120 mg daily dosage of 50:1 extract used as the standard by Commission E. Even if the consumer were taking a typical Ginkgo Biloba supplement of 200 mg. per day, a reasonable level of ORA consumption (1 to 2 bottles) would not create potential for toxicity.

Source of information:

The Complete German Commission E Monographs
Therapeutic Guide to Herbal Medicines
Published by: American Botanical Council
Austin, Texas
1998

Submitted by: Vancol Industries, Inc.
1700 E. 68th Ave.
Denver, CO 80229


Robert E. Yates Jr.
President

1999 NOV 30 P 12:25

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November 30, 1999

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

1999 DEC 13 P 2:35

GRAS Exemption claim for Chromium Picolinate used as an ingredient in a non-carbonated beverage.

The information which forms the basis of this claim may be obtained by the FDA from Vancol Industries, Inc at the address on the letterhead of this document.

Notified substance

Chromium Picolinate (98.0 – 100.0% by weight).

Proposed condition of use

Chromium Picolinate is proposed as an ingredient in a non-carbonated fruit beverage marketed under the brand name ORA. Distribution will achieve the widest consumer availability possible. Distribution channels will include but not be limited to grocery stores, convenience stores, mass merchandisers and drug store chains.

In ORA Citrus Punch 10 mcg of Chromium Picolinate is used per 8 oz. serving in a bottle that contains 20 fl. oz. or 2.5 servings. It is reasonable to expect that a consumer may drink 1 or more containers per day.

Chromium Picolinate is added to ORA Citrus Punch flavor because Chromium Picolinate enhances the bodies ability to generate energy.. Consumers have a tremendous interest in consuming products which can help them achieve higher levels of daily performance in their work and play and which can contribute to their overall health and wellbeing. Chromium Picolinate at the level of 10 mcg per serving safely helps meet those needs.

Scientific basis for GRAS determination

Material Safety Data Sheet as provided by Ashland Chemical Co., shows the following information:

000007

HAZARDS IDENTIFICATION

Swallowing

Swallowing small amounts of this material during normal handling is not likely to cause harmful effects. Swallowing large amounts may be harmful.

Target Organ Effects

Overexposure to this material (or its components) has been suggested as a cause of the following effects in humans: anemia.

Developmental Information

There are no data available for assessing risk to the fetus from maternal exposure to this material.

Cancer Information

There is no information available. The chance of this material causing cancer is unknown. This material is not listed as a carcinogen by the International Agency for Research on Cancer, the National Toxicology Program, or the Occupational Safety and Health Administration.

Exposure Guidelines

No exposure limits established.

Potential for overconsumption

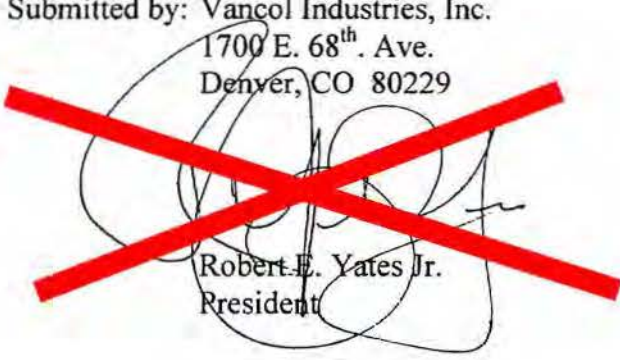
It is unlikely that a consumer would or could consume enough ORA Citrus Punch beverage to create an overdose of Chromium Picolinate. The two factors which negate potential for overconsumption are the fact that the MSDS indicates that the dosage required to create an adverse condition is quite high.. The second factor is the low level of Chromium Picolinate in each serving of product. Each 20 oz. bottle of ORA contains 25 mcg of Chromium Picolinate. The RDI for chromium is 120 mcg per day. A consumer would need to consume 4.8 bottles to ingest a 120 mcg daily dosage of Chromium Picolinate. Since the RDI tends to be a minimum level of consumption required to maintain adequate health the probable maximum level of ORA consumption (1 to 2 bottles daily) would not create potential for toxicity.

Source of information:

Ashland Chemical Co.

Submitted by: Vancol Industries, Inc.

1700 E. 68th Ave.
Denver, CO 80229



Robert E. Yates Jr.
President



January 18, 2000

Robert E. Yates, Jr.
President
Vancol Industries, Inc.
1700 E. 68th Avenue
Denver, CO 80229

Re: GRAS Notice (GRN) No. 000036

Dear Mr. Yates:

The Food and Drug Administration (FDA) has received the notice, dated December 20, 1999, that you 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)). FDA received this notice on January 3, 2000 and designated it as GRN No. 000036.

The subjects of your notice are chromium picolinate, *Ginkgo biloba* leaf extract, and *Panax ginseng* root extract. The notice informs FDA of the view of Vancol Industries, Inc. that chromium picolinate, *Ginkgo biloba* leaf extract, and *Panax ginseng* root extract are GRAS, through scientific procedures, for use as ingredients in a non-carbonated beverage or tea.

In accordance with proposed § 170.36(f), a copy of the information in your notice that conforms to the information described in proposed § 170.36(c)(1) is available for public review and copying on the Office of Premarket Approval's homepage on the World Wide Web. If you have any questions about your notice, please feel free to contact me at 202-418-3103.

Sincerely yours,

Lawrence J. Lin, Ph.D.
Division of Petition Control, HFS-215
Office of Premarket Approval
Center for Food Safety
and Applied Nutrition

(b) (5)

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Memorandum of Telephone Conversation

Date: January 28, 2000

Between: Lawrence Lin, Ph.D. (HFS-215)

and

John Ravnik, Vancol Industries, Inc. 303-289-8655

Subject: GRN #36, Chromium picolinate, Ginkgo biloba leaf extract,
and Panax ginseng root extract

On January 27, 2000, I telephoned Mr. Yates and was told by his staff that he was not available. Mr. Yates asked Mr. Ravnik to returned my call the next day. I indicated to him that we would like to receive the data and information that are the basis of their GRAS determinations for chromium picolinate, Ginkgo biloba leaf extract, and Panax ginseng root extract. Mr. Ravnik replied that they would provide the information to us as soon as possible.

Lawrence J. Lin, Ph.D.

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*talked to him
again 3/29*

000027

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Memorandum of Telephone Conversations

Date: December 2, 1999 and December 14, 1999

Between: Paulette Gaynor, Ph.D. (HFS-215)

and

Robert E. Yates, Jr. and John Ravnik, Vancol Industries, Inc., (303)289-8655

Subject: GRAS notice for ginseng extract and GRAS notice for *Gingko biloba* extract

On December 2, 1999, I telephoned Mr. Yates regarding a GRAS notice for ginseng extract and a GRAS notice for *Gingko biloba* leaf extract that he sent to the Office of Premarket Approval (OPA). I explained to Mr. Yates that OPA could not accept either submission as each lacked statements or information that distinguish a GRAS notice from a GRAS affirmation petition, and that these elements are in proposed 21 CFR 170.36(c)(1). I further explained that a difference between a GRAS notice and a GRAS affirmation petition is that for a GRAS notice a notifier takes responsibility for the GRAS determination, while for a GRAS affirmation petition a petitioner asks the Food and Drug Administration (FDA) to take responsibility for the GRAS determination. I then explained that we could not accept a GRAS notice unless a notifier takes responsibility for the GRAS determination and this statement would need to be in active voice and should read like the GRAS proposed rule (proposed 21 CFR 170.36(c)(1) which may be found on page 18961 of the proposed rule; 62 FR 18937; April 17, 1997).

Mr. Yates asked Mr. Ravnik to join in this telephone conversation. I then explained to Mr. Yates and Mr. Ravnik that proposed 21 CFR 170.36(c)(1), the GRAS exemption claim, would not be the appropriate place for detailed information or summary that is described under proposed 21 CFR 170.36(c)(2-4). I also explained that this particular telephone conversation would focus on the elements in the GRAS exemption claim, rather than on the detailed information or summary. Our focus on the detailed information or summary would occur later in this process (i.e., during our evaluation, which would occur after we file a notice).

For the GRAS exemption claim, we suggest that you follow proposed 21 CFR 170.36(c)(1). I explained that these elements (i.e., proposed 21 CFR 170.36(c)(1)(i-v)) generally are short statements. I then briefly explained the five elements under proposed 21 CFR 170.36(c)(1). Proposed 21 CFR 170.36(c)(1)(i) is the name and address of the notifier. Proposed 21 CFR

000024

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170.36(c)(1)(ii) is the common or usual name of the "notified substance." Proposed 21 CFR 170.36(c)(1)(iii) contains the applicable conditions of use. Proposed 21 CFR 170.36(c)(1)(iv) is the basis for the GRAS determination (i.e., through scientific procedures or through experience based on common use in food). A basis of common use in food pertains specifically to pre-1958 use in food. I also reminded Mr. Yates and Mr. Ravnik that proposed 21 CFR 170.36(c)(1)(iv) is a succinct statement of the basis. Proposed 21 CFR 170.36(c)(1)(v) is a statement that the data and information that are the basis for the notifier's GRAS determination are available for the FDA's review and copying at reasonable times at a specific address set out in the notice or will be sent to FDA upon request. I further explained that the intent of proposed 21 CFR 170.36(c)(1)(v) is that a notifier would agree to both procedures, and that depending on the circumstances, we might choose one procedure or the other. If a notifier doesn't agree to both procedures for making the data available to the agency, we would be unable to file the notice.

I also informed Mr. Yates and Mr. Ravnik that while we received three copies of the *Ginkgo biloba* submission, we received only two copies of the ginseng submission. I reminded Mr. Yates and Mr. Ravnik that in order to evaluate each submission, we would need three revised copies.

On December 14, 1999, I telephoned Mr. Yates regarding a GRAS notice for chromium picolinate that he sent to OPA. As Mr. Yates was not available, I left him a message explaining that OPA could not accept and file this submission for chromium picolinate as it lacks statements or information that distinguish a GRAS notice from a GRAS affirmation petition. I further explained that these deficiencies are similar to those in his two previous submissions (i.e., a GRAS notice for ginseng extract and a GRAS notice for *Ginkgo biloba* leaf extract) that we discussed on December 2, 1999.

Paulette M. Gaynor, Ph.D.

(b) (5)



000025



DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration
Memorandum of Telephone Conversation

Dates: March 13, 2000; March 27, 2000; and March 29, 2000

Between: Linda Kahl, Ph.D. (HFS-206)
and
Richard Mann, Keller and Heckman (202)434-4229

Subject: GRAS Notice GRN No. 000037 - Conversion of GRAS Petition GRP 1G0371
(Whey Protein Isolate and Dairy Product Solids)

I telephoned Mr. Mann regarding the view of the notifier, American Dairy Products Institute (ADPI), that both whey protein isolate and dairy product solids are GRAS for use "in foods." I asked Mr. Mann whether we should consider the use of these ingredients "in foods" to include foods subject to the statutes that the Food Safety and Inspection Service (FSIS) implements. Mr. Mann indicated that the notice for use in food should be as broad as possible and authorized me to send a copy of the notice to FSIS. Mr. Mann did qualify his authorization, however, by noting that he might reconsider if it appeared that the evaluation by FSIS would delay the response to his conversion request.

On March 27, 2000, I left a voice mail message for Mr. Mann regarding the statutory basis for the ADPI's view that both whey protein isolate and dairy product solids are GRAS. In both GRP 1G0371 and GRN 000037, ADPI states that the statutory basis is through experience based on common use in food. Given that the petition makes clear that both ingredients are prepared by processes that were not common in 1958, I told Mr. Mann that it appeared to FDA that his client had selected "common use in food" as the basis for the GRAS determination because both ingredients are components of food that was commonly consumed before 1958. I explained that FDA currently views the history of consumption of a food substance as a component of commonly consumed food to be an element of a scientific evaluation of safety. I also explained that the correspondence between ADPI and FDA during FDA's evaluation of GRP 1G0371 focused on a scientific evaluation of safety rather than on whether the ingredients had been consumed as such prior to 1958. Given these facts, I asked whether Mr. Mann would object to our evaluation of GRN 000037 on the basis of scientific procedures.

On March 29, 2000, Mr. Mann returned my call and concurred that the ingredients in question had been consumed as components of food prior to January 1, 1958, but had not been consumed as isolated components prior to that date. Therefore, he agreed that it would be appropriate to consider the consumption of these ingredients prior to 1958 as scientific evidence regarding their safety, and to have the agency evaluate all procedures.


Linda S. Kahl

Dated: 3/29/00

000015



DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service
Food and Drug Administration

Memorandum

Date March 13, 2000

From Linda S. Kahl, Ph.D., Office of Premarket Approval (HFS-206), Center for Food Safety and Applied Nutrition (CFSAN), Food and Drug Administration (FDA), 200 C Street, S.W., Washington, DC 20204

Subject GRAS Notice No. GRN 000037; Use of Whey Protein Isolate and Dairy Product Solids "in Food," including at least one identified use in meat products

To Robert Post, Ph.D., Labeling and Additives Policy Division (LAPD), Office of Policy, Program Development and Evaluation, Food Safety and Inspection Service, (FSIS) 300 12th Street, SW, Room 602, Washington, DC 20250-3700.

As per the draft Memorandum of Understanding between FDA/CFSAN and FSIS/LAPD, I am requesting consultation on GRAS Notice No. GRN 000037 for the use of whey protein isolate and dairy product solids. The notifier, American Dairy Products Institute (ADPI), has determined that these food ingredients are GRAS for use in foods. ADPI explicitly identifies one use of whey protein isolate in products subject to the Federal Meat Inspection Act - i.e., the use of whey protein isolate as a water binder in meat and sausage. On March 13, 2000, I spoke with the notifier's agent, Richard Mann of Keller and Heckman and asked whether we should consider the use of dairy product solids "in food" to include foods subject to the statutes that FSIS implements. Mr. Mann indicated that the notice for use in food should be as broad as possible and authorized me to send a copy of the notice to you.

The use of whey protein isolate and dairy product solids was originally the subject of a GRAS affirmation petition (GRP 1G0371). Consistent with FDA's proposal to replace the GRAS affirmation petition process with a notification procedure, ADPI has requested that FDA convert GRP 1G0371 to a GRAS notice.

I have enclosed the following materials:

1. A letter dated January 13, 2000, from Keller and Heckman (on behalf of ADPI), requesting that FDA convert GRP 1G0371 to a GRAS notice. This letter includes a "GRAS exemption claim" for whey protein isolate and dairy product solids. The GRAS exemption claim describes the conditions of use.
2. A letter dated January 10, 1995, from Keller and Heckman on behalf of ADPI regarding GRP 1G0371. In this letter, ADPI makes clear that they are requesting that FDA affirm the GRAS status of two food ingredients - whey protein isolate and dairy product solids.
3. The original GRAS affirmation petition, which included a total of three food ingredients. As explained in the letter dated January 10, 1995, ADPI considers that one of these original ingredients (i.e., lactose product) is subsumed within the ingredient "dairy product solids." The original petition lays out the petitioner's rationale for requesting GRAS affirmation.

000010

Page 2 - Robert Post, Ph.D.

I am requesting that FSIS/LAPD provide advice to FDA, in writing, on any criteria, restrictions, conditions of use, or prohibitions that FSIS/LAPD believes necessary concerning use of the substance in products subject to the Federal Meat Inspection Act or the Poultry Products Inspection Act.

Please direct your written response to my attention. If you have any questions, I can be reached by telephone at (202)418-3101; by telefax at (202)418-3131; or by electronic mail at LKAHL@BANGATE.FDA.GOV


Thank you.


Linda S. Kahl, Ph.D.

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Page 3 - Robert Post, Ph.D.

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000012



March 17, 2000

Richard F. Mann
Keller and Heckman, LLP
1001 G Street N.W.
Suite 500 West
Washington, DC 20001

Re: GRAS Notice (GRN) No. 000037

Dear Mr. Mann:

The Food and Drug Administration (FDA) has received the letter, dated January 13, 2000, that you submitted on behalf of the American Dairy Products Institute (ADPI). Your letter requests that FDA convert the filed GRAS affirmation petition GRP 1G0371 to a GRAS notice 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 conversion request on January 14, 2000 and designated it as GRN No. 000037.

Your conversion request relates to two direct human food ingredients: whey protein isolate and dairy product solids. Your conversion request informs FDA of the view of ADPI that whey protein isolate and dairy product solids are GRAS, through experience based on common use in food, for use in food. According to your conversion request, whey protein isolate would be used for nutritional purposes as a source of high quality protein in a wide variety of high-energy food and beverage products. Whey protein isolate would also be used for a variety of functional effects that are associated with proteins, e.g., as a gelation aid in yogurts and pudding, as a water binder in meat and sausage, as a foaming or whipping aid in toppings and fillings, and as an emulsifier in ice cream, margarine, and mayonnaise. According to your conversion request, dairy product solids would be used in a wide variety of foods in the production of alcohol, fermentation to organic chemicals, hydrolysis to galactose and glucose syrups, and sugar and corn syrup replacers.

As we discussed by telephone on March 13, 2000, you consider the intended use of whey protein isolate and dairy product solids "in foods" to include use in products that would be subject to statutes implemented by the U.S. Department of Agriculture, Food Safety and Inspection Service (FSIS). Consistent with that telephone conversation, we have sent a copy of GRN No. 000037, together with a copy of GRP 1G0371, to the Labeling and Additives Policy Division of FSIS.

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Page 2 - Mr. Mann

In accordance with proposed § 170.36(f), a copy of the information in your notice that conforms to the information described in proposed § 170.36(c)(1) is available for public review and copying on the Office of Premarket Approval's homepage on the World Wide Web. If you have any questions about the notice, please feel free to contact me at (202)418-3101.

Sincerely yours,

Linda S. Kahl, Ph.D.
Division of Product Policy, HFS-206
Office of Premarket Approval
Center for Food Safety
and Applied Nutrition

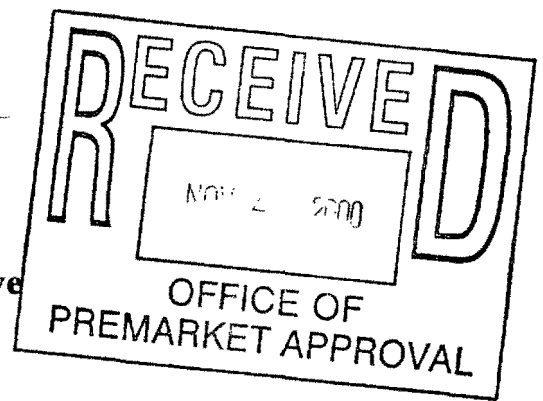
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000014



JHB
Justin Garrett Hill
26216 Alizia Canyon Drive
Calabasas, CA, 91302
213-840-0432



GRAS Notification and Premarket Approval Exemption

This notification is offered to show exemption from premarket approval for the use of the botanical extract Milk Thistle (*Silybum marianum*) as an ingredient in a malt based beverage and prove its status as "Generally Recognized As Safe."

Contents

1. Top 10 internet Health related information websites *p. 2*
2. Onhealth.com's compilation of information on Milk Thistle *p3 - p15*
3. Article from The Herb Quarterly (see highlighted parts) *p16 - p18*
4. Guidelines to the Good Manufacturing Practices of Milk Thistle extract preparation *p19 - p23*
from Homeopathic Preparation guidelines
5. Milk Thistle extract analysis and dosage *p 24*
6. Summary *p25* ~~*p26*~~

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Los Angeles Times

C10

MONDAY, APRIL 10, 2000

Top 10 Web Sites by Category

The PC Data Online Top 10 Hit Lists are based on traffic obtained from a sample panel of more than 100,000 U.S. home Internet users, balanced to represent the U.S. home Internet population. For the week ending April 1.

Entertainment

This week	Last week	Site	% of total Web audience reached	Unique users (millions)
1	1	Real.com	5.7%	3,058
2	2	ESPN.com	4.4	2,367
3	4	Shockwave.com	3.2	1,743
4	8	lgn.com	3.1	1,699
5	*	WindowsMedia.com	3.0	1,622
6	7	Ticketmaster.com	3.0	1,597
7	*	Abc.com	2.9	1,575
8	3	Disney.com	2.8	1,515
9	6	Mtv.com	2.7	1,443
10	9	Sony.com	2.6	1,427

Home and Food

1	1	ValuPage.com	2.0%	1,054
2	10	Furniture.com	1.1	590
3	3	Food.com	0.8	436
4	8	Cooking.com	0.8	406
5	5	Garden.com	0.7	362
6	4	Foodtv.com	0.6	322
7	2	Ourhouse.com	0.6	318
8	*	Netgrocer.com	0.5	259
9	7	MyCoupons.com	0.4	234
10	*	FurnitureFind.com	0.4	222

Travel

1	2	Mapquest.com	4.0%	2,178
2	1	Priceline.com	3.4	1,838
3	3	Digitalcity.com	2.8	1,494
4	4	Expedia.com	2.3	1,239
5	6	AOLTravel	2.2	1,170
6	5	Travelocity.com	2.0	1,070
7	7	Previewtravel.com	1.1	612
8	*	Travelscape.com	1.0	543
9	8	Lowestfare.com	0.9	465
10	10	AmericanExpress.com	0.8	430

Finance

This week	Last week	Site	% of total Web audience reached	Unique users (millions)
1	1	NextCard.com	3.8%	2,033
2	3	MarketWatch.com	2.6	1,385
3	2	AOLPersonalFinance	2.5	1,344
4	4	ETrade.com	1.5	790
5	7	Essential.com	1.4	773
6	6	Fidelity.com	1.3	710
7	8	Ameritrade.com	1.3	681
8	9	WellsFargo.com	1.2	665
9	*	Schwab.com	1.2	662
10	5	Quicken.com	1.1	592

Health and Family

1	1	Onhealth.com	4.3%	2,314
2	4	WebMD.com	2.6	1,430
3	3	AOLKidsOnly	2.3	1,234
4	2	Drkoop.com	1.9	1,047
5	5	AOLHealth	1.3	682
6	*	IntelliHealth.com	1.1	605
7	*	AllHerb.com	1.1	588
8	8	Drugstore.com	1.0	556
9	*	EDiets.com	1.0	545
10	6	PlanetRx.com	0.9	509

Sports

1	2	ESPN.com	4.4%	2,367
2	3	AOLSports	3.5	1,865
3	1	SportsLine.com	3.2	1,720
4	4	Cnnsl.com	1.8	969
5	5	Nascar.com	1.5	797
6	6	Nba.com	0.9	467
7	*	MajorLeagueBaseball.com	0.7	356
8	*	Nhl.com	0.6	346
9	9	Nfl.com	0.6	299
10	7	Foxsports.com	0.5	296

* Web site not on last week's Top 10 list.

Source: PC Data Online

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VIRAL LOAD: 75,000

Highlight >>> Keep your brood healthy and happy with OnHealth's Family channel.



- Home >
- Diseases & Conditions >
- Women >
- Family >
- Baby >
- Alternative
- Lifestyle >
- Food & Fitness >
- Library >
- Community >
- Shopping >

Home > Alternative > Herbal Index > M > Milk Thistle Fruit



Alternative

Herbal Index

Milk Thistle Fruit

Latin Name *Silybum marianum* (L.) Gaertner
Pharmacopeial Name Cardui mariae fructus
Other Names blessed milk thistle, St. Mary thistle

Overview

The milk thistle of commerce is a standardized preparation extracted from the fruits (seeds) of *Silybum marianum* (L.) Gaertn., Asteraceae (syn. *Carduus marianus* L.), a plant native to the Mediterranean. The leaves have been used since Greco-Roman times as an herbal remedy for a variety of ailments, particularly liver problems. Eclectic physicians in the United States in the latter nineteenth and early twentieth centuries acknowledged the clinical benefits of preparations from the milk thistle seeds (technically the fruits) for "Congestion of the liver, spleen, and kidneys ..." (Felter and Lloyd, 1983). It is widely used in German phytotherapy for "chronic hepatitis of all types," and especially for fatty liver (cirrhosis) associated with alcoholics (Weiss, 1988).

Milk thistle is an example of a preparation that is required to be in the standardized, concentrated form in order to fully convey the desired, in this case, hepatoprotectant, effects. Milk thistle preparations are usually standardized to a concentration of 70 to 80% of three flavonolignans (silibinin, silychristin, and silydianin), collectively known as silymarin. According to research conducted by the original manufacturer and primary researcher of milk thistle extract, Madaus AG of Cologne, Germany, this level of concentration of silymarin is required

Also See
[Symptom-to-Herb Checker](#)

[ID That Herb Quiz](#)



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OnHealth
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 • Home Delivery

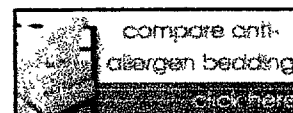
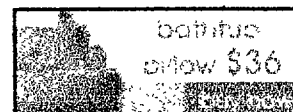
toolbox

• Symptom Checker
 • Symptom-to-Herb Checker
 • complete list

Shopping



[onhealthmarket.com](#)
 breathe happier and healthier



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Buy "Herbal

Medicine:

Expanded

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Monographs" from

amazon.com

order to enter into the bloodstream via the intestinal wall. Silymarin is poorly absorbed (20–50%) from the gastrointestinal tract; thus, the concentrated extract is recommended (Foster and Tyler, 1999; Robbers and Tyler, 1999).

The original product in Germany contains 70 mg silymarin. The Commission E approved uses and the subsequent use of milk thistle standardized extracts in the United States are based on a significant amount of chemical, pharmacological, and clinical research. There have been an estimated 120 clinical studies carried out on the proprietary milk thistle preparation from Madaus, known in Germany as Legalon[®]. A comprehensive and detailed review of the pharmacokinetics and clinical pharmacology of Legalon[®] has been published in English by the manufacturer (Anon., 1989).

Clinical studies suggest or confirm the efficacy of milk thistle extract for various hepatic disorders, including hepatitis A, alcoholic cirrhosis, and exposure to hazardous chemicals. Another relatively esoteric use is as a preventive and/or antidote to poisoning by the deathcap mushroom, *Amanita phalloides*. A preparation of the silibinin fraction is available in Germany as an intravenous (i.v.) drip for such acute cases.

A primary use for silymarin is in the treatment of liver damage due to ingestion of alcohol. An early double-blind study examined 66 patients, most with alcohol-induced toxic liver disease (Fintelmann and Albert, 1980). The 31 patients who received 420 mg/day of Legalon[®] showed a significant influence on serum levels of glutamic-oxalacetic transaminase (GOT), glutamic-pyruvic transaminase (GPT), and Gamma-GT over those 35 patients receiving placebo, with levels returning to normal more quickly in the treated group than placebo. Another double-blind study with 36 patients suffering from alcohol-induced liver disease found that pathological liver parameters (GOT, GPT, Gamma-GT, and bilirubin) were significantly reduced in the patients receiving silymarin (Legalon[®]) after six months of treatment compared to the placebo group (Feher et al., 1990). However, a multicenter study conducted in Spain from 1986 to 1989 found no effect for silymarin on the survival of patients or the clinical course of alcohol-induced

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randomized, controlled trial was performed to determine the effect of silymarin in the treatment of patients with alcohol- and non-alcohol-induced cirrhosis (Ferenci et al., 1989). Of the 170 patients, 87 received 420 mg of silymarin daily, compared with 83 placebo patients. The mean observation period was 41 months, with 10 dropouts in the placebo group and 14 in the treatment group. The four-year survival rate was $58 \pm 9\%$ in the silymarin-treated patients, and $39 \pm 9\%$ in the placebo group ($p=0.036$). No adverse side effects of drug treatment were observed.

In another double-blind, controlled study, the effects of silymarin on chemical, functional, and morphological alterations of the liver were examined (Salmi and Sarna, 1982). The 106 patients with liver disease in the study were selected on the basis of elevated serum transaminase levels. A total of 97 patients completed the four-week trial (47 treated and 50 placebo). A dose of 420 mg a day of silymarin produced a statistically significant greater decrease of GPT and GOT liver enzymes in the treated group than in the control. Serum total and bilirubin decreased more in the treated than in the control group, but the differences were not statistically significant.

Some forms of hepatitis have responded to silymarin treatment. A study reported on 67 subjects treated as outpatients for toxic metabolic liver damage, chronic hepatitis, and bile duct inflammation (Poser, 1971). After three months of treatment (525 mg/day of silymarin), chronic hepatitis was found to be significantly improved biotically. Conditions associated with bile duct inflammation also responded particularly well. Another double-blind study looked at the effect of silymarin in the treatment of acute viral hepatitis (Magliulo et al., 1978). A daily dose of 420 mg therapeutically influenced the increased serum levels of bilirubin, GOT, and GPT characteristically associated with acute viral hepatitis. After five days of treatment, the laboratory parameters regressed more in the treated group than for the placebo group. After three weeks, more patients in the treated group had attained normal values than in the placebo group. A statistical analysis showed a difference between GOT and bilirubin values in the silymarin and placebo groups, with a regression

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report of chronic infection by hepatitis B virus and hepatitis C virus demonstrated potential efficacy of treatment with milk thistle (10 g ground-up seeds in oatmeal with standardized (70%) milk thistle extract capsules three times daily) in combination with another herb known for its hepatoprotectant activity, *Phyllanthus amarus* (200 mg, three times daily) (McPartland, 1996).

In a review of important European clinical studies ranging from 1971 to 1988 (including those summarized above), the authors found the data suggests the effectiveness of silymarin not only in toxic and metabolic liver damage, but also in acute and chronic hepatitis (Hikino and Kiso, 1988). Silymarin's ability to stabilize the cell membrane and stimulate protein synthesis, while accelerating the process of regeneration in damaged liver tissue, was found to be important in its therapeutic efficacy.

The hepatoprotectant effect of milk thistle fractions (silymarin) is documented in other studies: these compounds can produce both a protective and curative effect on liver damage resulting from the highly toxic compounds phalloidin and alpha-amanitin (from the deathcap mushroom, *A. phalloides*). A multicenter trial conducted from 1979 to 1982 involved 220 cases of Amanita poisoning treated in German, Swiss, and Austrian hospitals (Hruby, 1984). Silibinin (administered i.v.) in supportive treatment was used. The mortality rate was 12.8%, compared to a mortality rate of 22.4% in a study where only 16 of the 205 patients were treated with 20 to 50 mg/kg/day of silibinin (Floersheim et al., 1982). Hruby concluded that the use of silibinin in addition to current methods of treating Amanita poisoning could lower mortality rates below any previously achieved.

A literature review noted that Legalon® is the best documented agent for the treatment of toxic liver impairment (Morazzani and Bombardelli, 1995). These authors also reviewed studies which suggest future use in dermatological and cosmetic products, based on a number of activities including promoting healing at wound sites, improved burn healing, and counteracting skin degeneration and aging via anti-inflammatory and free radical scavenging mechanisms. A more recent review concluded that despite some flaws

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in methodology of some of the clinical studies, Legalon[®] has not demonstrated adverse side effects and it "may be effective in improving the clinical courses of both acute and chronic viral, drug- and toxin-induced and alcoholic hepatitis" (Flora et al., 1998).

Because the well-documented antioxidant activity of silymarin has been shown to prevent lipoperoxidative hepatic damage by xenobiotic compounds (e.g., alcohol and certain pharmaceutical drugs), researchers attempted to determine whether milk thistle would be helpful for patients being administered psychotropic drugs. In a double-blind, placebo-controlled study, the efficacy of silymarin was evaluated in patients receiving psychotropic drugs as long-term therapy (Palasciano et al., 1994). Sixty women in the psychiatric ward of an Italian hospital were selected for the trial, all having been treated with either phenothiazines and/or butyrophenones for at least five years. They were randomly divided into four groups, with the silymarin patients receiving 800 mg a day for 90 days. Results showed that 800 mg/day of silymarin may be useful in the treatment of some instances of lipoperoxidative hepatic damage, such as the damage that may occur during long-term treatment with the psychotropic drugs.

Milk thistle extract has become increasingly popular in the United States as a dietary supplement for people with livers compromised by alcohol or exposure to toxic chemicals. Based on the increasing number of clinical studies indicating its safety and suggesting efficacy, consumers are also using this preparation as a means to ensure proper liver function and to assist in "detoxification" measures. Given this phytomedicine's well established safety and its reasonable documentation of efficacy, future clinical use of milk thistle extract should be explored as an adjunct therapy in chemotherapy to help offset the effects of powerful and potentially hepatotoxic conventional drugs.

U.S. pharmacopeial grade milk thistle consists of the dried ripe fruit of *S. marianum* with its pappus removed. It must contain not less than 2% of silymarin, calculated as silybin, as determined by a USP spectrophotometric assay method. Botanical identification must be confirmed by thin-layer chromatography (TLC) and macroscopic and microscopic examinations (USP

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24–NF 19, 1999). German pharmacopeial grade milk thistle also consists of the ripe fruit with the pappus removed. However, it must contain not less than 1.5% silymarin, calculated as silybin with reference to the dried drug. Total silymarin must be determined by a liquid chromatographic method (DAB method V.6.20.4). Botanical identity must be confirmed by TLC, macroscopic and microscopic examinations, and organoleptic evaluations. For example, the seed husk should have a bitter taste while the seed has an oleaginous taste, and it may not smell or taste rancid (DAB, 1997). The *German Homeopathic Pharmacopoeia* monograph requires that it contain not less than 1% of silymarin, calculated as silybin (GHP, 1993).

Description

Milk thistle fruit consists of ripe seed of *S. marianum* (L.) Gaertner [Fam. Asteraceae], freed from the pappus, and its preparations in effective dosage. The preparation contains silibinin, silydianin, and silychristin.

Chemistry and Pharmacology

Milk thistle seed contains 1.5–3% flavone lignans, collectively referred to as silymarin (Bruneton, 1995; Wichtl and Bisset, 1994); 20–30% fixed oil, of which approximately 60% is linoleic acid, approximately 30% is oleic acid, and approximately 9% is palmitic acid; 25–30% protein; 0.038% tocopherol; 0.63% sterols, including cholesterol, campesterol, stigmasterol, and sitosterol; and some mucilage (Meyer-Buchtela, 1999; Wichtl and Bisset, 1994). The three principle components of silymarin are the flavanolignans silybin, silychristin, and silidianin (Bruneton, 1995; Leung and Foster, 1996; Wichtl and Bisset, 1994).

The Commission E reported that silymarin acts as an antagonist in many experimental liver-damage models: phalloidin and amanitin (deathcap toxins), lanthanides, carbon tetrachloride, galactosamine, thioacetamide, and the hepatotoxic virus FV3 of cold-blooded vertebrates.

The therapeutic activity of silymarin is based on two sites or mechanisms of action:

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(a) *It alters the structure of the outer cell membrane of the hepatocytes in such a way as to prevent penetration of the liver toxin into the interior of the cell.*

(b) *It stimulates the action of nucleolar polymerase A, resulting in an increase in ribosomal protein synthesis, and thus stimulates the regenerative ability of the liver and the formation of new hepatocytes.*

Milk thistle extract provides hepatocellular protection by stabilizing hepatic cell membranes (McPartland, 1996). *Other actions include interruption of enterohepatic recirculation of toxins, stimulation of protein synthesis and regeneration of damaged hepatocytes, as well as antioxidant activity (McPartland, 1996).*

Recent research on silibinin and silichristin to promote faster regeneration of diseased liver tissue has focused on the ability of silibinin to stimulate the activity of the DNA-dependent RNA-polymerase I, causing an increase in rRNA synthesis and an accelerated formation of intact ribosomes. This results in a general increase in the rate of synthesis of all cellular proteins. *In vivo and in vitro* molecular modeling experiments indicate that silibinin may imitate a steroid hormone by binding specifically to polymerase I, thus stimulating enzyme activity (Sonnenbichler et al., 1998).

Uses

The Commission E approved the internal use of crude milk thistle fruit preparations for dyspeptic complaints. Formulations* are approved for toxic liver damage and for supportive treatment in chronic inflammatory liver disease and hepatic cirrhosis.

*[Ed. note: A "formulation" refers to an extract standardized to at least 70–80% silymarin, the collective name for the three compounds listed in the Description section.]

The German Standard License for milk thistle seed tea infusion indicates its use for digestive disorders, particularly for functional disturbances

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Contraindications

None known.

Side Effects

Crude preparation: None known.

Formulations: A mild laxative effect has been observed in occasional instances.

Use During Pregnancy and Lactation

No restrictions known.

Interactions with Other Drugs

None known.

Dosage and Administration

Unless otherwise prescribed: 12–15 g per day of powdered seed for making infusions and other *galenical formulations to be taken by mouth*. Formulations (e.g., dry extract noted below) equivalent to 200–400 mg per day of silymarin, calculated as silibinin.

For liver diseases:

Dry extract 40–70:1 (w/w), 70–80% silymarin: Swallow one capsule containing 100–200 mg of silymarin, twice daily in the morning and evening. Swallow with sufficient amounts of fluid; or, take one capsule containing approximately 140 mg of silymarin, two to three times daily (Brown, 1996).

For digestive disorders:

Decoction: Place approximately 3.0 g seed in 150 ml cold water, bring to a boil and simmer for 20 to 30 minutes, three to four times daily (Wichtl and Bisset, 1994).

Infusion: Steep approximately 3.5 g seed in 150 ml boiled water for 10 to 15 minutes, three to four times daily one half-hour before meals (Braun et al., 1997; Meyer-Buchtela, 1999; Wichtl and

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[Note: Silymarin is poorly soluble in water; teas have been analyzed with only about 10% of the original levels of silymarin from the fruits. Thus, for hepatic benefits, the concentrated extract is recommended (Foster and Tyler, 1999).]

Tincture: 15–25 drops, four to five times daily (Lust, 1974); 1–2 ml, three times daily (Hoffmann, 1992).

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Note

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1) The Overview section is new information.

2) Description, Chemistry and Pharmacology, Uses, Contraindications, Side Effects, Interactions with Other Drugs, and Dosage sections have been drawn from the original work. Additional information has been added in some or all of these sections, as noted with references.

3) The dosage for equivalent preparations (tea infusion, fluidextract, and tincture) have been provided based on the following example: Unless otherwise prescribed: 2 g per day of [powdered, crushed, cut or whole] [plant part]

Infusion: 2 g in 150 ml of water

Fluidextract 1:1 (g/ml): 2 ml

Tincture 1:5 (g/ml): 10 ml

4) The References and Additional Resources sections are new sections. Additional Resources are not cited in the monograph but are included for research purposes.

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Milk Thistle: Nature's Liver Protector

By Michael Castleman

Mainstream medicine has little to offer those with disease of the liver. "Most liver treatment," says herbal medicine authority Varro Tyler, Ph.D., the Lily distinguished professor of pharmacognosy (natural product medicine) at Purdue University, is simply supportive." Doctors keep patients comfortable and away from liver-damaging drugs, alcohol, and viruses until the organ can heal itself (if it can).

However, liver healing could be significantly spurred by a remarkable herb that has been hiding in plain sight for almost 2,000 years. It's milk thistle (*Silybum marianum*). This common herb's value against liver disease has been demonstrated in more than 100 rigorous scientific experiments. Unfortunately, the vast majority of these studies have been European, mostly German, and few mainstream American physicians read German botanical medicine journals. As a result, they are in the dark about milk thistle's astonishing liver-protective powers.

Mary's Milk

Milk thistle is native to the Kashmir region of India and Pakistan, but now grows throughout the temperate world. The plant grows from five to ten feet tall, and has large prickly leaves and reddish purple flowers with sharp spines that resemble artichokes. When de-spined, milk thistle leaves are edible, and some vegetable gardeners cultivate the plant as a substitute for spinach. When broken or crushed, the stems and leaves exude a milky white juice, hence this herb's name. Milk thistle's specific name, *marianum*, comes from an ancient legend that its leaf veins turned white after being touched by a drop of the Virgin Mary's breast milk.

Milk thistle has been used in traditional herbal medicine since the first century, when the Roman naturalist, Pliny the Elder (A.D. 23-79), wrote that the plant's milky juice was good for "carrying off bile." (Today "bile" denotes a product of the gall bladder, part of the liver, which assists in the digestion of fats, but in ancient times, bile was used more generally to describe any internal fluid.) The noted 16th century British herbalist, John Gerard, was the first to recommend milk thistle for liver problems, though his prescription was oblique. He actually suggested the herb for "expelling melancholy," which physicians at the time considered a liver ailment. Half a century later, Britain's most famous herbalist, Nicholas Culpepper, was the first to recommend milk thistle specifically for liver disorders. By the 19th century, German physicians were using a tincture prepared from milk thistle seeds (actually the plants seed like fruits) to treat jaundice and other liver diseases. America's 19th century eclectic physicians, who specialized in botanical medicines, adopted the herb for liver ailments and for intestinal cleansing.

With the rise of the modern pharmaceutical industry, U.S. research of herbal medicines declined considerably. Fortunately, this did not happen in Germany, where in 1949, scientists noticed that milk thistle seemed to protect animal livers from poisoning with highly toxic carbon tetrachloride. In 1968, scientists isolated the three specific liver-protective molecules in milk thistle - silibinin, silidianin, and silicristin - now known collectively as silymarin [milk thistle extract].

Studies Galore

More than 100 studies have confirmed silymarin's [milk thistle extract] liver-protective value. Here is a brief overview of what researches have discovered:

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* **Alcoholic Cirrhosis.** A 1989 report in the *Journal of Hepatology* (study of the liver) described a study involving 170 people with advanced alcoholic cirrhosis, an often fatal condition, and the nation's 11th leading cause of death, claiming 25,000 lives each year. The study participants were divided into two groups. One received 200 mg of milk thistle extract (140 mg of silymarin [milk thistle extract]) three times a day, the other received a medically inactive look-alike placebo. Both groups were followed for four years. During that time, the death rate in the placebo group was about 60 percent, but

among those taking silymarin [milk thistle extract], only 40 percent died, a highly statistically significant difference. Other studies have shown that silymarin [milk thistle extract] provides similar benefits for people suffering from cirrhosis.

*** Death cap mushroom poisoning.** The common wild mushroom, *Amanita phalloides*, is known as the "death cap" for a good reason. It takes only a handful of this widely distributed fungus to kill an adult, less to kill a child. Standard medical treatment - activated charcoal - is not particularly effective. Amanita mushroom ingestion proves fatal in about half of the cases. Twenty years ago, pilot studies showed that silymarin [milk thistle extract] treatment substantially reduced amanita-poisoning deaths in animals fed the mushroom. Subsequently, several human studies were launched. In one German hospital test, 60 consecutive people with amanita poisoning were given intravenous silymarin [milk thistle extract]. None died. Other studies have produced results that are similar, though not as spectacular. (However, the success of silymarin [milk thistle extract] in treating amanita poisoning should *not* encourage anyone to go mushroom hunting without training in amanita avoidance. Unless you are an experienced hunter, the only place to pick mushrooms is at a produce market.)

*** Hepatitis.** Hepatitis means liver inflammation. It is not one disease, but several, most of which are caused by different viruses that attack liver cells. The three most common forms are hepatitis A, B, and C. Hepatitis A is food borne. Hepatitis B and C are blood borne and sexually transmitted. Mainstream medicine treats all forms of hepatitis with rest and avoidance of alcohol and other drugs and toxins that tax the liver.

However, silymarin [milk thistle extract] is a more effective approach. In one study, 77 people with hepatitis were divided into two groups, one treated with silymarin [milk thistle extract], the other with a placebo. Average recovery time for the placebo-takers was 43 days, but those who took silymarin [milk thistle extract] recovered in an average of just 29 days.

*** Gallstones.** Up to 10 percent of Americans are estimated to have gallstones, little pebbles that develop in the gallbladder. Some cause no symptoms, but many cause abdominal pain, sometimes severe enough to require surgical removal of the gallbladder. Most gallstones are formed from cholesterol, and then precipitates out as stones. A low-fat, low-cholesterol diet helps prevent gallstones. So does silymarin [milk thistle extract]. In one study, people with gallstones were given 420 mg of silymarin [milk thistle extract] a day. Without diet changes, after several weeks, they showed significant reductions in the cholesterol concentration of their bile, which minimized the risk of stone formation.

*** Liver Function Tests.** The liver metabolizes all drugs, and powerful medications often stress the liver, producing abnormal liver function tests that sometimes require physicians to stop drug treatment people need. Silymarin [milk thistle extract] helps normalize liver function, allowing those who must take liver-harming medications to do so with less risk of liver damage. In one study, 66 women taking anticonvulsant or psychiatric medications showed abnormal liver-function tests. They began taking silymarin [milk thistle extract] in addition to their medication, and 52 of them showed significant improvements in liver function.

*** Occupational Toxic Chemical Exposure.** Like drugs, toxic chemicals also stress the liver, causing liver-function tests to register abnormal results. European studies show that silymarin [milk thistle extract] renormalizes liver-function tests in workers who produce pesticides, and in those exposed to toxic heavy metals, for example, lead and cadmium.

*** Psoriasis.** A few European studies suggest that silymarin [milk thistle extract] may even help treat the scaly skin patches of psoriasis.

How Silymarin [milk thistle extract] works

Silymarin [milk thistle extract] works in three ways. It strengthens the outer membranes of liver cells, preventing penetration by liver-damaging substances. This accounts for its effectiveness against amanita mushroom poisoning. Both silymarin [milk thistle extract] and the mushroom toxins bind to the same sites on liver cell membranes. As silymarin [milk thistle extract] blood levels increase, the milk thistle extract occupies the cell-membrane receptor sites, displacing the amanita toxins.

Silymarin [milk thistle extract] also protects liver cells because of its powerful antioxidant action. Antioxidants neutralize cell damage caused by chemically unstable oxygen molecules formed by high-fat diets, smoking, and other toxic substances. The best known antioxidants are Vitamin A (beta-carotene), Vitamin C, Vitamin E, and the mineral selenium. However, in the liver, silymarin [milk thistle extract] is more than 10 times as potent an antioxidant as Vitamin E.

Finally, silymarin [milk thistle extract] inhibits the action of the enzyme largely responsible for inflammation in

As far as scientists know, silymarin [milk thistle extract] does not interfere with the liver's metabolism of drugs, so it does not interfere with the action of medications.

Preventive Medicine?

You don't have to munch amanita mushrooms to stress your liver. Every day we're exposed to pollutants, pesticides, food additives, and other substances that the liver must detoxify. In addition, anyone who drinks alcohol or takes any medication - either prescription or over-the-counter drugs - boosts the liver's workload, and damages some liver cells in the process. Fortunately for all of us, the liver is quite large. It's the second largest organ, after the skin, so you can lose millions of liver cells and still function normally. But why lose even a single liver cell if you don't have to?

Recently, Scandinavian researchers tested silymarin's [milk thistle extract] effect on livers that were stressed but not seriously diseased. They selected 106 consecutive patients who had abnormal liver-function tests from alcohol use, but who did not have cirrhosis. Half took silymarin [milk thistle extract]; the other half received a placebo. After four weeks, the placebo group showed no change in liver-function, but the silymarin [milk thistle extract] group showed highly significant improvement, in some cases, complete normalization of liver-function, despite their alcohol consumption. Perhaps we all should take silymarin [milk thistle extract]. Robert McCaleb, president of the *Herb Research Foundation* in Longmont, Co., does: "If I worked in an occupation [that stressed the liver], I would take milk thistle regularly, once each workday morning. [but I don't, so] I take two tablets before working with paints or solvents, and I never take aspirin acetaminophen (tylenol) without also taking milk thistle. Finally, I always take milk thistle along when traveling because almost invariably I find myself at a cocktail party" (Sage Counsel).

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These supplementary guidelines are intended to highlight certain aspects that have a bearing on the manufacture of herbal medicinal products. Since medicinal plant materials are often obtained from varied geographical and commercial sources it may not always be possible to ascertain the conditions to which they may have been subjected. Criteria for their testing should take into consideration as many factors as possible based on experience with the material obtained from the source in question. The presence of a complexity of active substances is not uncommon in herbal medicinal products which renders the quantitative estimation of individual active principles difficult. Testing criteria should then be appropriately modified to accommodate special needs.

While the Genral Good Manufacturing Practices (GMP) Guideline 4th edition is applicable to the manufacture of herbal medicinal products, the information provided here should facilitate compliance with the GMP Division of the Food and Drug Regulations by manufacturers and importers of herbal medicinal products. Herbal medicinal products intended for parenteral use must be manufactured in accordance with the requirements outlined under STERILE PRODUCTS (C.02.029) of the general guidelines on Good Manufacturing Practices.

GLOSSARY OF TERMS

*See p 23
first*

The following additions to supplement the definitions provided under the Glossary of Terms in the general guidelines for Good Manufacturing Practices may be useful to manufacturers and importers of herbal medicinal products. The definitions given below apply to the terms used in these guidelines. They may have different meanings in other contexts:

- **HERBAL MEDICINAL PRODUCT:** A term generally applicable to the finished product and pertains to a medicinal product containing plant materials and/or plant preparations as its active ingredients.
- **MARKERS:** Generally employed to estimate the quantity of plant material or plant preparation in the finished product when constituents with known therapeutic activity are either not found or are uncertain. Markers are chemically defined constituents of a medicinal plant material utilised for control purposes.
- **MEDICINAL PLANT:** A plant, either cultivated or growing wild, utilised for its medicinal value.
- **MEDICINAL PLANT MATERIAL:** Either the whole plant or parts of medicinal plants in the crude state.
- **PLANT PREPARATIONS:** Herbal ingredients present in a form other than the crude medicinal plant material including powdered plant material, balsams, dried and fluid extracts, tinctures; essential oils, fatty oils, gums and resins, expressed juices prepared from plant material, and preparations obtained by fractionation, purification or concentration, without chemically defined isolated constituents regardless of whether or not its therapeutically active constituents have been identified.

PREMISES

1. Premises prevent the entry of insects, animals, fungus and extraneous terrestrial material. Suitable measures are in place to prevent their migration within other areas of the building should they be introduced into the storage area with the plant material.
2. Medicinal plant materials are stored in separate areas that are well ventilated.
3. Facilities for exhaust, collection and disposal are provided in areas where dust, vapours or fumes are normally generated.
4. Storage containers are placed in a manner that allow free circulation of air.
5. Where required, protection from light is provided and monitored.

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PERSONNEL

1. Individuals engaged in quality control are conversant with herbal terminology and have expertise in:

- 1.1 the identification of medicinal plant materials;
- 1.2 the recognition of possible adulterants, terrestrial contaminants, fungal and insect infestations;
- 1.3 the determination of non-uniformity within and differences in quality between consignments of medicinal plant materials.

2. Personnel working in storage areas are apprised of, and provided appropriate training in the handling of, materials that are when exposed, highly active, allergenic or otherwise hazardous.

SANITATION

See general interpretation.

RAW MATERIAL TESTING

1. Specifications for medicinal plant materials include the following:

- 1.1 botanical identity, including genus, species and authority (e.g., Linnaeus);
- 1.2 detailed information on:
 - 1.2.1 geographical source;
 - 1.2.2 cultivation and collection techniques;
 - 1.2.3 time of harvest;
 - 1.2.4 biological age (e.g., of bark)
 - 1.2.5 nature and extent of artificial fertilisers, pesticides, herbicides, insecticides and fumigants, etc., if used;
 - 1.2.6 nature and extent of radioactive residues, if applicable
- 1.3 the specific part (e.g., leaf, root, flower, etc.) if only a part of the plant is utilised and the general proportion of each if a mixture of parts is used;
- 1.4 when a dried plant is obtained from a supplier, system and conditions of drying are specified;
- 1.5 morphological and microscopical descriptions;
- 1.6 tests for identification including where appropriate, tests for known active ingredients or markers;
- 1.7 assay, where appropriate, of constituents of known therapeutic activity or markers;

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1.8 test methods and acceptable limits for:

1.8.1 moisture;

1.8.2 potentially toxic elemental contaminants e.g. lead, mercury, arsenic, etc.;

1.8.3 admixed foreign materials and adulterants;

1.8.4 fungal and/or microbial contamination;

1.8.5 mycotoxins;

2. Wherever appropriate, in addition to the above, specifications for plant preparations include the following:

2.1 test for identification;

2.2 test method and acceptable limits for particle size distribution for powdered plant material.

Note: Where a plant preparation containing a mixture of powdered plant materials are procured directly, evidence from the supplier to the effect that each plant material in the composite has been individually tested, and where the preparation is a liquid extract or tincture, the vehicle or solvent used and the conditions employed in their preparation should be documented.

MANUFACTURING CONTROL

1. Master formulae include the following:

1.1 A statement of the principal equipment to be used, including those employed in operations such as drying, comminution, size separation, extraction, concentration, etc.;

1.2 Detailed stepwise processing instructions including, where applicable;

1.2.1 checks on materials;

1.2.2 pretreatment such as drying, comminution, size separation, etc., for medicinal plant materials;

1.2.3 sequence for adding materials;

1.2.4 duration of extraction, concentration, mixing, etc., and the physical state of the preparation at the end of these processes.

2. Processing of fresh starting materials from vegetable origin commence as soon as possible after harvesting.

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2.1. Where processing cannot be initiated within few hours, harvesting is not undertaken under damp weather conditions and adequate precautions are taken to ensure that plants are not wet or are covered in dew when gathered.

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2.2. Where processing cannot be initiated prior to 8 hours after harvesting, starting materials are stored under conditions that are appropriate for the conservation of the medicinal plant, preferably refrigerated and used within 48 hours.

2.3. Organic or inorganic waste material are removed from the plant material during harvesting.

2.4. If the starting material is a bark, intact piece(s) and if a root is used the whole plant or a complete or partial aerial segment with root attached, should accompany the consignment.

QUALITY CONTROL DEPARTMENT

See general interpretation.

PACKAGING MATERIAL TESTING

See general interpretation.

FINISHED PRODUCT TESTING

1. Specifications are of pharmacopoeial or equivalent status and in compliance with the marketing authorization.

2. In addition to the tests that are considered critical to the quality of the specific dosage form, specifications normally include appropriate qualitative tests and

2.1 wherever feasible, tests for the quantitative determination of each active ingredient, or a joint determination of several active ingredients;

2.2 where the therapeutic activity of constituents is known, test for its determination or when this is not feasible specifications are based on the determination of markers;

2.3 tests for microbial content.

RECORDS

See general interpretation.

SAMPLES

See general interpretation. Samples of starting material and finished product are retained under conditions that are appropriate for their conservation.

STABILITY

1. Data are available over the proposed shelf-life and under the labelled storage conditions.

2. Stability protocol incorporates all critical quality tests.

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2.1 In addition to the determination of the stability of constituents with known therapeutic activity, to the extent feasible tests are designed to establish that other substances present

are stable

2.2 where the determination of stability of each active ingredient in a herbal medicinal product containing several medicinal plant materials or plant preparations is difficult, the stability of the product is determined by appropriate methods overall methods of assay and physical or sensory tests or other appropriate tests.

2.3 levels of preservatives and stabilizers are monitored or when these are not present in the formulation, alternative tests which provide an assurance that the product is self preserving over its shelf-life.

Note: Certain aspects of the chart outlined in the general guidelines, particularly the tests pertaining to physical characteristics may be useful in designing a stability protocol. However, this chart is intended only as a guide and does not preclude the inclusion of other tests which may be pertinent to the quality of the herbal medicinal product under investigation.

**GOOD MANUFACTURING PRACTICES
SUPPLEMENTARY GUIDELINES
FOR HOMEOPATHIC PREPARATIONS
FINAL VERSION**

October 1996

*This page should
be p19.*

PREFACE

These supplementary guidelines are intended to elaborate certain aspects that have relevance to the manufacture of homeopathic preparations. Homeopathic preparations do not generally lend themselves to the application of conventional analytical methodology. This necessitates the exercise of rigorous vigilance during their manufacture in order to ensure absolute purity and reproducibility. Since in the realm of homeopathic philosophy efficacy is intricately linked to the process of preparation thereby making efficacy synonymous with quality, the importance of attention to detail in the execution and documentation of every stage of manufacture cannot be overemphasized.

The information provided here when placed in context with the general guidelines for Good Manufacturing Practices (GMP) should facilitate compliance with the GMP Division of the Food and Drug Regulations by manufacturers and importers of homeopathic preparations. Homeopathic preparations intended for parenteral use must be manufactured in accordance with the requirements outlined under STERILE PRODUCTS (C.02.029) of the general guidelines for Good Manufacturing Practices.

GLOSSARY OF TERMS

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The following additions to supplement the definitions provided under the Glossary of Terms in the general guidelines for Good Manufacturing Practices may be useful to manufacturers and importers of homeopathic preparations. The definitions given below apply to the terms used in this supplementary

Milk Thistle Extract Analysis and Dosage

The liquid extract of Milk Thistle is made from isolated crushed Milk Thistle seed husks and pure grain alcohol. The finished extract contains approximately 100mg of Silymarin (the active compound in Milk Thistle) per 5ml of 66% pure grain alcohol solution. The extract is produced in accordance with the Good manufacturing Practices of Herbal medicinal products.

The finished Milk Thistle extract is mixed with a traditional style Malt based beverage (2 row malt, nugget hops, munich malt, carmel malt 6-90) in a finishing tank directly prior to bottling. Each 355ml glass bottle serving will contain approximately 3.5 ml of Milk Thistle Extract (70mg Silymarin).

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Summary

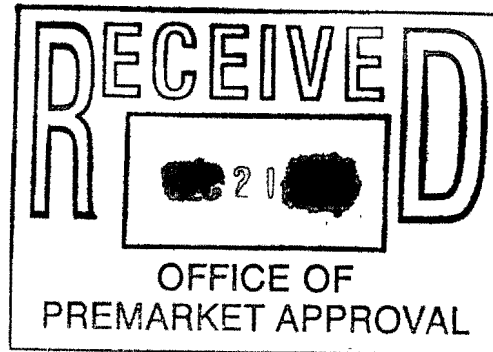
Milk Thistle has been proved in hisological (biopsy), clinical and laboratory data to be safe and non-toxic with no known drug interactions and shows no indication of the ability to overdose. Milk Thistle is accepted by the scientific community as being beneficial to the liver through a variety of pathways and is clearly a positive factor in the human body.

11/16/2000



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213-840-0432

December 14, 2000

This notification is offered to show exemption from the premarket approval requirements of the Federal Food and Drug Administration for the use of 2.5ml of the botanical extract Milk Thistle (*Silybum marianum*, 2.5ml extract = 50 mg silymarin) as an ingredient per 355ml serving of a malt based beverage (beer), and prove its status as "Generally Regarded As Safe" based on scientific procedures as determined by JHB Inc.

Milk Thistle Extract Preparation

Brucia Plant Extracts, Inc., a leader in quality botanical preparations since 1975, produce the Milk Thistle extract.

Personnel

1. All individuals engaged in quality control are conversant with herbal terminology and have expertise in:

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- 1.1 the identification of Milk Thistle
 - 1.2 the recognition of possible adulterants, terrestrial contaminants, fungal and insect infestations
 - 1.3 the determination of non-uniformity within the differences in quality between consignments of plant materials.
2. Personnel working in storage areas are apprised of, and provided appropriate training in the handling of raw materials

Raw Material Testing

1. Specifications for medicinal plant materials include the following:

- 1.1 botanical identity, including genus, species and authority(e.g., *Silybum marianum*);
- 1.2 detailed information on
 - 1.2.1 geographical source;
 - 1.2.2 cultivation and collection techniques;
 - 1.2.3 time of harvest;
 - 1.2.4 biological age
 - 1.2.5. nature and extent of artificial fertilizers, pesticides, herbicides, insecticides and fumigants, etc., if used;
 - 1.2.6 nature and extent of radioactive residues, if applicable

see p 20

1.2.7 where processing cannot be initiated prior to 8 hours after harvesting, Milk Thistle seeds are stored under conditions that are appropriate for conservation

Chemistry and Pharmacology

Milk Thistle seeds contain 1.5-3% flavonolignans, collectively referred to as silymarin (Bruneton, 1995; Wichtl and Bisset, 1994); 20-30% fixed oil, of which approximately 60% is linoleic acid, approximately 30% is oleic acid, and approximately 9% is palmitic acid; 25-30% protein; 0.038% tocopherol; 0.63% sterols. Including cholesterol, campesterol, stigmasterol, and sitosterol; and some mucilage (Meyer-Buchtela, 1999; Wichtl and Bisset, 1994). The three principle components of silymarin are the flavanolignans silybin, silychristin, and silidianin (Bruneton, 1995; Leung and Foster, 1996; Wichtl and Bisset, 1994).

see p8

Liquid Extract

The Milk Thistle extract is produced using a Maceration/Percolation process with certified organic isolated crushed Milk Thistle seed husks (seed freed from pappus), pure grain alcohol, and purified water in accordance with the Good Manufacturing Practices of Herbal medicinal products. The finished liquid Milk Thistle extract consists of approximately 100 mg silymarin (as determined by USP spectrophotometric assay method) per 5ml of 66% pure grain alcohol.

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The liquid extract is stored in sterilized glass containers, in a cool, dark environment appropriate for conservation.

Use of Liquid Milk Thistle Extract

The finished Milk Thistle extract is used as an ingredient in a traditional style malt based beverage (2 row malt, nugget hops, munich malt, carmel malt 6-90). The Milk Thistle extract is mixed with the brewed beverage in a finishing tank directly prior to bottling. Each 355ml glass bottle serving will contain approximately 2.5 ml of Milk Thistle extract (50 mg silymarin). The Milk Thistle Extract is used as a flavor enhancer, particularly as a bittering agent.

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Overview

The milk thistle of commerce is a standardized preparation extracted from the fruits (seeds) of *Silybum marianum* (L.) Gaertn., Asteraceae (syn. *Carduus marianus* L.), a plant native to the Mediterranean. The leaves have been used since Greco-Roman times as an herbal remedy for a variety of ailments, particularly liver problems. Eclectic physicians in the United States in the latter nineteenth and early twentieth centuries

see p3

acknowledged the clinical benefits of preparations from the milk thistle seeds (technically the fruits) for "Congestion of the liver, spleen, and kidneys ..." (Felter and Lloyd, 1983). It is widely used in German phytotherapy for "chronic hepatitis of all types," and especially for fatty liver (cirrhosis) associated with alcoholics (Weiss, 1988).

Milk thistle is an example of a preparation that is required to be in the standardized, concentrated form in order to fully convey the desired, in this case, hepatoprotectant, effects. Milk thistle preparations are usually standardized to a concentration of 70 to 80% of three flavonolignans (silibinin, silychristin, and silydianin), collectively known as silymarin. According to research conducted by the original manufacturer and primary researcher of milk thistle extract, Madaus AG of Cologne, Germany, this level of concentration of silymarin is required to survive degradation by gastric fluids and in order to enter into the bloodstream via the intestinal wall. Silymarin is poorly absorbed (20-50%) from the gastrointestinal tract; thus, the concentrated extract is recommended (Foster and Tyler, 1999; Robbers and Tyler, 1999). p3

The original product in Germany contains 70 mg silymarin. The Commission E approved uses and the subsequent use of milk thistle standardized extracts in the United States are based on a significant amount of chemical, pharmacological, and clinical research. There have been an estimated 120 clinical studies carried out on the proprietary milk thistle preparation from Madaus, known in Germany as Legalon®. A comprehensive and detailed review of the pharmacokinetics and clinical pharmacology of Legalon® has been published in English by the manufacturer (Anon., 1989). pf

Clinical studies suggest or confirm the efficacy of milk thistle extract for various hepatic disorders, including hepatitis A, alcoholic cirrhosis, and exposure to hazardous chemicals. Another relatively esoteric use is as a preventive and/or antidote to poisoning by the deathcap mushroom, *Amanita phalloides*. A preparation of the silibinin fraction is available in Germany as an intravenous (i.v.) drip for such acute cases. pf

A primary use for silymarin is in the treatment of liver damage due to ingestion of alcohol. An early double-blind study examined 66 patients, most with alcohol-induced toxic liver disease (Fintelmann and Albert, 1980). The 31 patients who received 420 mg/day of Legalon® showed a significant influence on serum levels of glutamic-oxalacetic transaminase (GOT), glutamic-pyruvic transaminase (GPT), and Gamma-GT over those 35 patients receiving placebo, with levels returning to normal more quickly in the treated group than placebo. Another double-blind study with 36 patients suffering from alcohol-induced liver disease found that pathological liver parameters (GOT, GPT, Gamma-GT, and bilirubin) were significantly reduced in the patients receiving pf

silymarin (Legalon®) after six months of treatment compared to the placebo group (Feher et al., 1990). In another study, a randomized, controlled trial was performed to determine the effect of silymarin in the treatment of patients with alcohol- and non-alcohol-induced cirrhosis (Ferenci et al., 1989). Of the 170 patients, 87 received 420 mg of silymarin daily, compared with 83 placebo patients. The mean observation period was 41 months, with 10 dropouts in the placebo group and 14 in the treatment group. The four-year survival rate was 58+/-9% in the silymarin-treated patients, and 39+/-9% in the placebo group (p=0.036). No adverse side effects of drug treatment were observed.

In another double-blind, controlled study, the effects of silymarin on chemical, functional, and morphological alterations of the liver were examined (Salmi and Sarna, 1982). The 106 patients with liver disease in the study were selected on the basis of elevated serum transaminase levels. A total of 97 patients completed the four-week trial (47 treated and 50 placebo). A dose of 420 mg a day of silymarin produced a statistically significant greater decrease of GPT and GOT liver enzymes in the treated group than in the control. Serum total and bilirubin decreased more in the treated than in the control group, but the differences were not statistically significant. p5

Some forms of hepatitis have responded to silymarin treatment. A study reported on 67 subjects treated as outpatients for toxic metabolic liver damage, chronic hepatitis, and bile duct inflammation (Poser, 1971). After three months of treatment (525 mg/day of silymarin), chronic hepatitis was found to be significantly improved bioptically. Conditions associated with bile duct inflammation also responded particularly well. Another double-blind study looked at the effect of silymarin in the treatment of acute viral hepatitis (Magliulo et al., 1978). A daily dose of 420 mg therapeutically influenced the increased serum levels of bilirubin, GOT, and GPT characteristically associated with acute viral hepatitis. After five days of treatment, the laboratory parameters regressed more in the treated group than for the placebo group. After three weeks, more patients in the treated group had attained normal values than in the placebo group. A statistical analysis showed a difference between GOT and bilirubin values in the silymarin and placebo groups, with a regression of GPT values in favor of silymarin. A recent case report of chronic infection by hepatitis B virus and hepatitis C virus demonstrated potential efficacy of treatment with milk thistle (10 g ground-up seeds in oatmeal with standardized (70%) milk thistle extract capsules three times daily) in combination with another herb known for its hepatoprotectant activity, Phyllanthus amarus (200 mg, three times daily) (McPartland, 1996). § In a review of important European clinical studies ranging from 1971 to 1988 (including those summarized above), the authors found the data suggests the effectiveness of silymarin not only in toxic and metabolic liver damage, but also in acute and chronic hepatitis p5 p6 (Hikino and Kiso, 1988). Silymarin's ability to stabilize the cell membrane and

stimulate protein synthesis, while accelerating the process of regeneration in damaged liver tissue, was found to be important in its therapeutic efficacy.

The hepatoprotectant effect of milk thistle fractions (silymarin) is documented in other studies: these compounds can produce both a protective and curative effect on liver damage resulting from the highly toxic compounds phalloidin and alpha-amanitin (from the deathcap mushroom, *A. phalloides*). A multicenter trial conducted from 1979 to 1982 involved 220 cases of *Amanita* poisoning treated in German, Swiss, and Austrian hospitals (Hruby, 1984). Silibinin (administered i.v.) in supportive treatment was used. The mortality rate was 12.8%, compared to a mortality rate of 22.4% in a study where only 16 of the 205 patients were treated with 20 to 50 mg/kg/day of silibinin (Floersheim et al., 1982). Hruby concluded that the use of silibinin in addition to current methods of treating *Amanita* poisoning could lower mortality rates below any previously achieved. p6

A literature review noted that Legalon® is the best-documented agent for the treatment of toxic liver impairment (Morazzani and Bombardelli, 1995). These authors also reviewed studies which suggest future use in dermatological and cosmetic products, based on a number of activities including promoting healing at wound sites, improved burn healing, and counteracting skin degeneration and aging via anti-inflammatory and free radical scavenging mechanisms. A more recent review concluded that despite some flaws in methodology of some of the clinical studies, Legalon® has not demonstrated adverse side effects and it "may be effective in improving the clinical courses of both acute and chronic viral, drug- and toxin-induced and alcoholic hepatitis" (Flora et al., 1998). p6

Because the well-documented antioxidant activity of silymarin has been shown to prevent lipoperoxidative hepatic damage by xenobiotic compounds (e.g., alcohol and certain pharmaceutical drugs), researchers attempted to determine whether milk thistle would be helpful for patients being administered psychotropic drugs. In a double blind, placebo-controlled study, the efficacy of silymarin was evaluated in-patients receiving psychotropic drugs as long-term therapy (Palasciano et al., 1994). Sixty women in the psychiatric ward of an Italian hospital were selected for the trial, all having been treated with either phenothiazines and/or butyrophenones for at least five years. They were randomly divided into four groups, with the silymarin patients receiving 800 mg a day for 90 days. Results showed that 800 mg/day of silymarin may be useful in the treatment of some instances of lipoperoxidative hepatic damage, such as the damage that may occur during long-term treatment with the psychotropic drugs. p7

The therapeutic activity of silymarin is based on two sites or mechanisms of action:

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(a) It alters the structure of the outer cell membrane of the hepatocytes in such a way as to prevent penetration of the liver toxin into the interior of the cell. (b) It stimulates the action of nucleolar polymerase A, resulting in an increase in ribosomal protein synthesis, and thus stimulates the regenerative ability of the liver and the formation of new hepatocytes.

p 8

Milk thistle extract provides hepatocellular protection by stabilizing hepatic cell membranes (McPartland, 1996). Other actions include interruption of enterohepatic recirculation of toxins, stimulation of protein synthesis and regeneration of damaged hepatocytes, as well as antioxidant activity (McPartland, 1996).

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Recent research on silibinin and silichristin to promote faster regeneration of diseased liver tissue has focused on the ability of silibinin to stimulate the activity of the DNA-dependent RNA-polymerase I, causing an increase in rRNA synthesis and an accelerated formation of intact ribosomes. This results in a general increase in the rate of synthesis of all cellular proteins. In vivo and in vitro molecular modeling experiments indicate that silibinin may imitate a steroid hormone by binding specifically to polymerase I, thus stimulating enzyme activity (Sonnenbichler et al., 1998).

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Contraindications

None known.

Side Effects

None known.

Formulations: A mild laxative effect has been observed in occasional instances.

Interactions with Other Drugs

None known.

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Summary

Milk Thistle extract has become increasingly popular in the United States as a dietary supplement. Based on the existing and increasing number of clinical studies indicating its safety and suggesting efficacy, JHB Inc. recognizes 2.5 ml of Milk Thistle extract (50 mg silymarin) per serving as GRAS and complimentary when added to a malt based beverage.

December 14, 2000



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Note

This material was adapted from *The Complete German Commission E Monographs- Therapeutic Guide to Herbal Medicines and on health with webmd*. M. Blumenthal, W.R. Busse, A. Goldberg, J. Gruenwald, T. Hall, C.W. Riggins, R.S. Rister (eds.) S. Klein and R.S. Rister (trans.). 1998. J. Hill 2000.

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January 19, 2001

Mr. Justin Hill
JHB Inc.
26216 Alizia Cyn. Drive
Calabasas, CA 91302

Re: GRAS Notice (GRN) No. 000066

Dear Mr. Hill:

The Food and Drug Administration (FDA) has received the notice, dated December 14, 2000, that you submitted on behalf of JHB Inc., 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 January 3, 2001 and designated it as GRN No. 000066.

The subject of your notice is milk thistle extract. The notice informs FDA of the view of JHB Inc., that milk thistle extract is GRAS, through scientific procedures, for use as a flavor enhancer in a malt-based beverage not to exceed good manufacturing practices.

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 your notice, contact Dr. Lawrence Lin at 202-418-3103.

Sincerely yours,

Suzette Williams
Division of Product Policy, HFS-205
Office of Premarket Approval
Center for Food Safety
and Applied Nutrition

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Memorandum

Date	March 29, 2001
From	Consumer Safety Officer
Subject	GRAS Notice (GRN) 000066
To	Administrative File, GRN 000066

This memorandum summarizes the notice dated December 14, 2000 that Mr. Justin Hill of JHB Inc. 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 Premarket Approval (OPA) received the notice on January 3, 2001, and designated it as GRN 000066.

The subject of the notice is milk thistle extract. The notice informs FDA of the view of JHB Inc. that milk thistle extract is GRAS, through scientific procedures, for use as a bittering agent in a malt-based beverage, where a 355 milliliter glass bottle will contain approximately 2.5 milliliter of the extract.

I. Data and information that JHB presents in GRN 000066**A. Identity of Milk Thistle Extract**

In GRN 000066, JHB describes milk thistle extract as a standardized extract containing 100 milligrams silymarin (a collective name for the three flavonolignans; silybin, silychristin, and silidianin) per 5 milliliter of 66 percent ethanol (described as pure grain alcohol). The extract is not otherwise characterized. JHB does not provide specifications to describe the food grade material.

B. Manufacturing of Milk Thistle Extract

In GRN 000066, JHB states that milk thistle extract is made from isolated crushed seed husks and pure grain alcohol. No specific manufacturing conditions are provided other than saying that it is produced in accordance with good manufacturing practices of herbal medicinal products. JHB indicates that milk thistle extract will be obtained from Brucia Plant Extracts, Inc., a firm that has produced botanical preparations since 1975.

C. Intended Conditions of Use and Use levels

In GRN 000063, JHB informs FDA that the intended use of milk thistle extract is as a bittering agent in a malt-based beverage, where a 355 milliliter glass bottle will contain approximately 2.5 milliliter of the extract. Thus, the 355 milliliter glass bottle will contain approximately 50 milligrams of silymarin.

D. Estimated Dietary Exposure

JHB gives no intake estimates for milk thistle extract.

E. Safety Information on Milk Thistle Extract**000052**

In GRN 000066, JHB presents a review article about milk thistle extract that was posted on the Internet (i.e., www.onhealth.com). There is no indication that this review article has been published in a peer-reviewed scientific journal. This article discusses several clinical studies focused on the efficacy (the liver protective effect) of milk thistle extract. JHB relies solely on this article for the safety discussion on the intended use of milk thistle extract.

II. Evaluation by the Office of Premarket Approval

The notice relies solely on clinical studies that focus on the efficacy of milk thistle as a drug to treat patients with liver disease. These clinical studies differ from studies designed for food additives, which are typically conducted to assess toxicity in experimental animals for the purpose of extrapolating the results to the general population. Drugs are used by specific groups within the population for specific periods of time under medical supervision, while foods (including drinks) may be consumed by all without restriction or medical supervision. Further, the safety evaluation of a drug use considers both benefits and risks to patients, while that of a food use considers only potential risks that might arise from human consumption. Therefore, studies that only address the efficacy of milk thistle as a drug are inadequate to evaluate its safety as a food ingredient.


The general absence of reports of adverse effects regarding the medicinal uses of milk thistle, as is claimed in the notice, does not provide sufficient evidence to conclude that the use of milk thistle in food is safe. One published article (Adverse Drug Reactions Advisory Committee, "An Adverse Reaction to the Herbal Medication Milk Thistle," *Medical Journal of Australia*, **170**, 218-219 (1999)) reports a 57-year-old woman with a two-month history of intermittent episodes of sweating, nausea, colicky abdominal pain, fluid diarrhea, vomiting, weakness and collapse, associated with the use of milk thistle capsules. Allergic reactions associated with use of milk thistle are also reported in the literature. A recent article (Barnes et al., "Different Standards for Reporting ADRs to Herbal Remedies and Conventional OTC Medicines," *British Journal of Clinical Pharmacology*, **45**, 496-500 (1998)) indicates that adverse drug reactions to herbal medicines are more likely to be under-reported than those to conventional over-the-counter medicines.

As described in the notice, a bottle of beer (355 ml) with 2.5 ml of milk thistle extract would have 50 mg of silymarin, a substance known to produce effects in the liver. Given this exposure, we would expect the notice to include the results of extensive tests in animals to support safety, e.g., genotoxicity studies, chronic feeding studies in more than one species of laboratory animal, etc., in addition to a discussion of the data and information available in humans. The notice should also address the absorption, metabolism, distribution, and elimination of milk thistle and its potential to interfere with human metabolism as part of an overall safety assessment strategy. As appropriate, the results of such animal tests could be used to establish a No-Observed-Effect-Level (NOEL) in order to determine the acceptable daily intake (ADI), or level of consumption of milk thistle extract that would be safe for the general population. The notice neither provides information about a NOEL established in any oral toxicological study nor provides reference to an ADI that has been established through such a study.


III. Conclusions

We have evaluated the information in GRAS Notice No. GRN 000066. In light of the issues described above, the notice does not provide a sufficient basis for a determination that milk thistle extract is safe under the proposed conditions of use.

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Lawrence J. Lin, Ph.D.

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The references used to compile the monograph on **MILK THISTLE above ground parts** were:

- 2** Blumenthal M, et al. ed. The Complete German Commission E Monographs: Therapeutic Guide to Herbal Medicines. Trans. S. Klein. Boston, MA: American Botanical Council, 1998.
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Monograph

NATURAL MEDICINES
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Also Known As Cardui mariae fructus, Holy Thistle, Lady's Thistle, Marian Thistle, Mary Thistle, Our Lady's Thistle, St. Mary Thistle, Silybum, Silymarin.

CAUTION: See separate listings for Milk Thistle above ground parts and Blessed Thistle.

Scientific Names Silybum marianum, synonym Carduus marianum.
Family: Asteraceae or Compositae.

People Use This For Orally, the fruit and seed of milk thistle are used for dyspeptic complaints (2), as a liver protectant (6,2613), treating toxic liver damage caused by chemicals (6,2614), Amanita mushroom poisoning (6,2615), supportive therapy for chronic inflammatory liver disease and hepatic cirrhosis (2,2616), chronic hepatitis (795), loss of appetite, liver and gallbladder complaints, and diseases of the spleen (8,18). Intravenously, the seed and fruit are used as a supportive treatment for Amanita phalloides mushroom poisoning (795). Historically, the fruit and seed are roasted for use as a coffee substitute (11).

Safety POSSIBLY SAFE ...when used appropriately (2,12,512,795). **PREGNANCY AND LACTATION:** Insufficient reliable information available; avoid using.

Effectiveness POSSIBLY EFFECTIVE ...when used orally for dyspeptic complaints (2), treating toxic liver damage, supportive treatment of chronic inflammatory liver disease and hepatic cirrhosis (2), alcoholic liver disease (6,795,2618), drug-induced liver disease, bile duct inflammation, and chronic hepatitis (6,795). ...when used intravenously (IV) as supportive treatment for liver damage due to Amanita phalloides mushroom poisoning (6,7,795). Clinical studies of milk thistle's effectiveness have used formulations standardized to 70% silymarin and used an average dose of 200-400 mg daily. There is insufficient reliable information available about the effectiveness of milk thistle fruit and seed for their other uses.

Mechanism of Action The milk thistle fruit is also referred to as the seed. Silymarin, a milk thistle fruit and seed extract complex, consists of four flavanolignans, silibinin (silybin), isosilybinin, silichristin (silychristin), and silidianin. These exhibit liver-protective and antioxidant effects (6). The therapeutic activity of silymarin is based on two mechanisms of action. The first is an alteration of the outer hepatocyte cell membrane that prevents toxin penetration, and the second involves the stimulation of nucleolar polymerase A resulting in increased ribosomal protein synthesis, which can stimulate liver regeneration and the formation of new hepatocytes (2). Silymarin undergoes

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	<p>enterohepatic recirculation and has higher concentrations in liver cells (6). In vitro, Silymarin inhibits liver damage from chemicals, drugs, alcohol, viruses, and the <i>Amanita phalloides</i> mushroom toxin (6,795). Silymarin can have benefits in alcohol-induced liver disease and in acute viral and chronic hepatitis (6,795). Silymarin decreases insulin resistance in people with alcoholic cirrhosis (2617). Intravenous silibinin greatly improves human survival in cases of <i>Amanita phalloides</i> mushroom poisoning (6,7,795).</p>
Adverse Reactions	<p>The fruit and seed of milk thistle taken orally can cause an occasional laxative effect (2,795). There is one reported case of a woman who experienced intermittent episodes of sweating, nausea, abdominal pain, vomiting, diarrhea, weakness and collapse, requiring hospitalization (3525). Mild allergic reactions can occur with milk thistle use (6). It can cause an allergic reaction in individuals sensitive to the Asteraceae/Compositae family. Members of this family include ragweed, chrysanthemums, marigolds, daisies, and many other herbs.</p>
Interactions with Herbs & Supplements	<p>Insufficient reliable information available.</p>
Interactions with Drugs	<p>ASPIRIN: Theoretically, altered aspirin metabolism in individuals with liver cirrhosis might be improved with concomitant use of milk thistle (19). HEPATOTOXIC DRUGS: Silymarin can help prevent liver damage caused by drugs, including butyrophenones, phenothiazines, phenytoin, acetaminophen, alcohol, and halothane (19). CISPLATIN: Theoretically, concomitant administration of the constituent, silibinin, might help prevent kidney damage (19). OTHER DRUGS: There's preliminary evidence that milk thistle fruit and seed can inhibit the cytochrome P450 (CYP450) 3A4 enzyme (6450). Theoretically, milk thistle fruit and seed might increase levels of drugs metabolized by CYP450 3A4. However, so far, this interaction has not been reported in humans. Some drugs metabolized by CYP450 3A4 include lovastatin (Mevacor), ketoconazole (Nizoral), itraconazole (Sporanox), fexofenadine (Allegra), triazolam (Halcion), and numerous others. Use milk thistle fruit and seed cautiously or avoid in patients taking these drugs.</p>
Interactions with Foods	<p>No interactions are known to occur, and there is no known reason to expect a clinically significant interaction with milk thistle fruit and seed.</p>
Interactions with Lab Tests	<p>LIVER ENZYMES: Silymarin decreases elevated serum transaminase levels and test results (2618).</p>
Interactions with Diseases or Conditions	<p>CROSS-ALLERGENICITY: Can cause an allergic reaction in individuals sensitive to the Asteraceae/Compositae family. Members of this family include ragweed, chrysanthemums, marigolds, daisies, and many other herbs.</p>
Dosage and Administration	<p>ORAL: Clinical studies of milk thistle's effectiveness have used the standardized 70% silymarin extract at a daily dose of 200-400 mg calculated as silibinin (2,7,8). Alternatively, the typical dose is 12-15 grams of the dried fruit or seed per day (8). Some people make a milk thistle tea, but the active</p>

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ingredients are not very soluble in water (8,515).

INTRAVENOUS: For *Amanita phalloides* mushroom poisoning, the common dose is 20-50 mg/kg over 24 hours, divided into four infusions, each administered over a two hour period. This is usually started within 48 hours after mushroom ingestion (6,7). Intravenous silibinin is unavailable in the US.

Comments

The broken leaves of the milk thistle plant exude of milky sap, and the plant was once grown in Europe as a vegetable for salads and as a substitute for spinach (6). Avoid confusion of the seed and fruit with blessed thistle (*Cnicus benedictus*) or the other parts of milk thistle.

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MILK THISTLE above ground parts

Also Known As Cardui mariae herba, Holy Thistle, Lady's Thistle, Marian Thistle, Mary Thistle, St. Mary Thistle, Silymarin.

CAUTION: See separate listings for Milk Thistle seed and Blessed Thistle.

Scientific Names Silybum marianum, synonym Carduus marianum.
Family: Asteraceae or Compositae.

People Use This For Orally, the above ground parts of the milk thistle plant are used for maintaining health, stimulating and treating dysfunction of the gallbladder and liver, and for treating jaundice, pleurisy, and diseases of the spleen (2). Historically, the parts have been used for treating malaria, uterine complaints, and stimulating menstrual flow (18). For food use, the plant is grown in Europe as a vegetable for salads and as a substitute for spinach (6).

Safety **LIKELY SAFE** ...when consumed in amounts commonly found in food (11). There is insufficient reliable information available about the oral medicinal use of the milk thistle above ground parts. **PREGNANCY AND LACTATION:** Insufficient reliable information available; avoid using.

Effectiveness There is insufficient reliable information available about the effectiveness of the above ground parts of the milk thistle plant (2).

Mechanism of Action A milk thistle plant extract enhances estradiol binding to estrogen receptors, induces transcription activity in estrogen-responsive cells, and enhances estradiol-induced transcription activity in estrogen-responsive cells (6180).

Adverse Reactions Milk thistle can cause an allergic reaction in individuals sensitive to the Asteraceae/Compositae family. Members of this family include ragweed, chrysanthemums, marigolds, daisies, and many other herbs.

Interactions with Herbs & Supplements Insufficient reliable information available.

Interactions with Drugs No interactions are known to occur, and there is no known reason to expect a clinically significant interaction with milk thistle above ground parts.

Interactions with Foods No interactions are known to occur, and there is no known reason to expect a clinically significant interaction with milk thistle above ground parts.

Interactions with Lab Tests No interactions are known to occur, and there is no known reason to expect a clinically significant interaction with milk thistle above ground parts.

Interactions with Diseases **CROSS-ALLERGENICITY:** Can cause an allergic reaction in individuals sensitive to the Asteraceae/Compositae family.

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or Conditions	Members of this family include ragweed, chrysanthemums, marigolds, daisies, and many other herbs. HORMONE SENSITIVE CANCERS/CONDITIONS: Because milk thistle might have estrogenic effects (6180), women with hormone sensitive conditions should avoid milk thistle. Some of these conditions include breast, uterine, and ovarian cancer, and endometriosis and uterine fibroids.
Dosage and Administration	ORAL: The typical dose of milk thistle is one cup of the tea two to three times daily (18). The tea is prepared by steeping 1/2 teaspoon of the above ground parts in 150 mL boiling water for 5-10 minutes and then straining.
Comments	The broken leaves of the milk thistle plant exude a milky sap. Avoid confusion with milk thistle seed. There is limited information available about the above ground parts of the milk thistle plant.

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An adverse reaction to the herbal medication milk thistle (*Silybum marianum*)

There is widespread use of herbal and other complementary medicines in Australia.¹ Many people believe that these products are "natural" and therefore free from side effects, but this is not necessarily the case. Various adverse reactions can occur.² A recent publication has suggested that adverse drug reactions to herbal remedies are even more under-reported than those to conventional over-the-counter (OTC) medicines.³ The Adverse Drug Reactions Advisory Committee (ADRAC) receives and analyses reports of adverse drug reactions to complementary medicines as well as to prescribed and OTC medications, and has published reports on adverse reactions to royal jelly, chaparral and Kombucha tea.⁴⁻⁷

Case

A report of a severe reaction in association with milk thistle has recently been received. A 57-year-old woman presented with a two-month history of intermittent episodes of sweating, nausea, colicky abdominal pain, fluid diarrhoea, vomiting, weakness and collapse. The episodes could last up to 24 hours, but she felt completely well between attacks. The episodes were not related to food or to any obvious activity. She had been taking ethinyloestradiol and amitriptyline.

The patient had no abnormalities on examination, with a regular pulse rate of 80 beats/minute and only a 6 mmHg postural drop in blood pressure. Neurological examination was normal. Differential diagnoses considered were pheochromocytoma, carcinoid syndrome and thyrotoxicosis. She was admitted to hospital for investigation one day after an attack. Investigations revealed an initial minor elevation of urea and haemoglobin level, and raised white cell count, which were believed to be due to dehydration and reverted to normal without therapy. All other tests, including thyroid function, blood glucose level, urinary free catechol level and 5-hydroxyindolacetic acid levels, were normal and her erythrocyte sedimentation rate was 15 mm/hour.

She was then questioned further about any changes to her routine in the previous two months. She admitted that she had started taking Microgenics Herbals Milk Thistle Vegicaps (Aust L 56929; Optimum Healthcare Pty Ltd) for headaches and liver cleansing exactly two months previously. On the day before admission to hospital she had taken a capsule a few hours before the onset of symptoms. On reflection, she thought that all the attacks had occurred after taking the capsules. She ceased taking milk thistle and had no further symptoms. A few weeks later she took another capsule and experienced a violent reaction similar to the one causing hospital admission.

Comment

Milk Thistle Vegicaps contain *Silybum marianum* (commonly known as milk thistle), a plant which is native to southern Europe, southern Russia, Asia Minor and North Africa. It now grows naturally in Australia, but the drug is largely obtained from cultivated plants. The active constituents of *Silybum marianum* fruit include a group of flavonolignans known collectively as silymarin.⁸ Silymarin consists of four isomers, with silybin accounting for 50% of the total. These substances have been studied both *in vitro* and *in vivo* and found to have antioxidant properties and to protect against light-induced skin cancer.^{9,10} They are also hepatoprotective in rodents. In humans, silymarin has been used to protect against poisoning with the toxic mushroom *Amanita phalloides*, and as both prophylaxis and treatment for liver disease.¹¹ Silymarin has been studied in a number of prospective clinical trials.^{12,13} Its efficacy in liver disease is still debated, but a recent overview indicated that no serious side effects have been reported.¹⁴

The present case report describes a severe and time-associated reaction to milk thistle capsules confirmed on rechallenge. It is, however, quite possible that the problem was caused not by silybin, but by some other substance contained in the capsules. Drew and Myers point out a number of ways in which medications can cause problems because of extrinsic effects unrelated to the intended active ingredient.² These idiosyncratic reactions are just as likely to occur with complementary medicines as with more conventional medications. ADRAC has received only two previous reports in association with milk thistle. In one, an 83-year-old man was found to be thrombocytopenic. The relation with taking milk thistle was uncertain. In the other report, a woman developed abdominal pains, nausea, listlessness and insomnia after taking milk thistle.

The important message for health professionals is to take a full drug history from patients, particularly when unusual symptoms occur. It is necessary to ask directly about herbal and alternative substances as well as prescribed and OTC medications. If there is any suspicion that an adverse drug reaction has occurred it should be reported to ADRAC on a "blue card", where it will be reviewed by the Committee, collated and compared with other reactions related to complementary medicines.

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Adverse Drug Reactions Advisory Committee, PO Box 100, Woden, ACT 2606



Book Reviews

Choices for the dying

Dying well: a holistic guide for the dying and their carers. Richard Reoch. Rydalmere: Hodder & Stoughton 1997 (191 pp., \$29.95). ISBN: 0 7336 0345 9.

Different explanatory models of illness are adopted by varied cultures. Some of these have given rise to complementary therapies used in our community, particularly at times of stress. *Dying well* describes a variety of complementary therapies which may be of benefit to the dying and their carers. Drawing frequently on Buddhist, shiatsu, Tibetan and Chinese *chi kung* traditions, it is simply written for the lay public, with illustrations on each page. It would be easily read by the very ill. Its death talk is direct, reassuring and normalising of the dying process and is likely to promote an adaptive adjustment for those struggling to accept their predicament.

The strength of the book lies in its use of existential and spiritual approaches to aid the dying. Its weakness is its failure to integrate any of the principles and treatments of Western medical culture into its recommendations. In the latter sense, it is not truly holistic. It narrowly describes doctors as obliged to treat, cure and prolong life.

While offering a comprehensive range of complementary therapies, it omits effective and evidence-based approaches to symptom relief. For example, many patients will need more than olive, gentian or gorse to relieve their depression. If followed literally, some of the advice could be dangerous. However, if the wise reader smiled at aromatherapy approaches, such as a bath of ginger or rosemary to relieve constipation, then the overall approach that the book adopts to deal with death anxiety would be beneficial.

When practitioners are asked to recommend a book on complementary therapies, they can be reassured that this book does not include outrageous practices and will help to promote courage, dignity and a calm inner peace in those confronting their own or a loved one's death.

David W Kissane

Director, University of Melbourne Centre for Palliative Care
Kew, VIC

Joint review

Rheumatology highlights 1997. Paul Emery (editor). Oxford: Health Press 1998 (88 pp., US\$19.95). ISBN: 1 899541 12 8.

Confronted and often confounded by the plethora of new research publications, physicians and general practitioners will find this publication a useful means of remaining cognizant of important current laboratory and clinical research in rheumatology. The decision made by the editor, Professor Paul Emery, an eminent UK rheumatologist, to address specific highlights rather than attempt a comprehensive summary of 1997 rheumatic disease research has resulted in a slim, affordable volume of 88 pages which succinctly summarises key areas and can be comfortably scrutinised in 60-90 minutes. The book is "reader friendly", with colour-coded headings and chapters. Boxed summaries of topic highlights in each chapter cleverly link what would otherwise be a collection of independent, sometimes unrelated, topics.

The 11 chapters are written by practitioners who have contributed to the specific area of research under discussion and, not unexpectedly, there is an emphasis on rheumatoid arthritis that reflects the main research interest of the editor. Topics range from the cellular conundrum that dominates "the pathogenesis of rheumatoid arthritis" to the more clinical and topical issues regarding the use of combination therapy and minocycline in rheumatoid arthritis.

As promised on the front cover, this book provides "fast facts", and I felt that editorial input in the form of additional chapter comments would have added further, possibly provocative, interest. Professor Emery proposes further annual reviews, and I would like to see topics relating to pathogenesis and management of bone loss in rheumatic disease and chondroprotection in the 1998 issue. However, the main challenge for the editor will be to maintain the standard of contributions provided in this issue.

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Caroline Brand

Rheumatologist, Melbourne, VIC

Different standards for reporting ADRs to herbal remedies and conventional OTC medicines: face-to-face interviews with 515 users of herbal remedies

Joanne Barnes,¹ Simon Y. Mills, Neil C. Abbot,² Martin Willoughby² & Edzard Ernst¹

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Aims To determine whether adverse drug reactions (ADRs) to herbal remedies would be reported differently from similar ADRs to conventional over-the-counter (OTC) medicines by herbal-remedy users.

Methods Face-to-face interviews (using a structured questionnaire) with 515 users of herbal remedies were conducted in six pharmacy stores and six healthfood stores in the UK. The questionnaire focused on the likely course of action taken by herbal-remedy users after experiencing an ADR associated with a conventional OTC medicine and a herbal remedy.

Results Following a 'serious' suspected ADR, 156 respondents (30.3%) would consult their GP irrespective of whether the ADR was associated with the use of a herbal remedy or a conventional OTC medicine, whereas 221 respondents (42.9%) would not consult their GP for a serious ADR associated with either type of preparation. One hundred and thirty-four respondents (26.0%) would consult their GP for a serious ADR to a conventional OTC medicine, but not for a similar ADR to a herbal remedy, whereas four respondents (0.8%) would consult their GP for a serious ADR to a herbal remedy, but not for a similar ADR to a conventional OTC medicine. Similar differences were found in attitudes towards reporting 'minor' suspected ADRs.

Conclusions Consumers of herbal remedies would act differently with regard to reporting an ADR (serious or minor) to their GP depending on whether it was associated with a herbal remedy or a conventional OTC medicine. This has implications for herbal pharmacovigilance, particularly given the increasing use of OTC herbal remedies. The finding that a high proportion of respondents would not consult their GP or pharmacist following ADRs to conventional OTC medicines is also of concern.

Keywords: herbal medicines, drugs, nonprescription, drug monitoring, adverse drug reaction reporting systems, alternative medicine

Introduction

In the UK, herbal remedies (or 'phytomedicines') are increasingly being used by the general public on a self-selection basis to replace or complement conventional medicines [1]. The use of herbal remedies is widespread across Europe—in 1991, the total over-the-counter (OTC) market for herbal remedies was £1.45 billion [2]. Another source estimated that, in 1992, the herbal market of the European Community was worth US\$2.4 billion [3]. More recently, the market for licensed herbal medicines in the UK was estimated to be worth £38 million in 1996, representing over half of the total market for complementary remedies [4].

One of the reasons for the popularity of herbal remedies is the belief among many users and suppliers of herbal remedies that these preparations are natural and therefore

'safe' [1]. This, however, is a misconception—herbal remedies can produce adverse drug reactions (ADRs) [5, 6], some of which can be serious and even fatal [7, 8]. However, because users believe that such remedies are 'safe', individuals experiencing ADRs may not associate these with their use of herbal remedies [7]. A further complication is that, in the UK, the majority of herbal remedies are self-prescribed [1], and many individuals may be reluctant to tell their general practitioner (GP) that they are using them [7]. Even if ADRs are reported by patients, their GPs may not be fully briefed about the use and effects (adverse or otherwise) of herbal remedies.

The European Union (EU) has commissioned research into this problem through its BIOMED (Biomedical and Health Research) programme. As part of that programme, this study was designed to determine whether ADRs to herbal remedies would be reported differently from similar ADRs to conventional OTC medicines, and to identify experiences of ADRs to herbal remedies and how they are perceived by consumers.

Correspondence: Joanne Barnes, Department of Complementary Medicine, Postgraduate Medical School, University of Exeter, 25 Victoria Park Road, Exeter EX2 4NT.

Methods

Customer interview

Experienced interviewers, recruited and trained for this task by a market research company, were provided with study questionnaires, photographs and lists of examples of herbal remedies, other complementary remedies and conventional OTC medicines, and a list of examples of ADRs. These materials were used to assist interviewers and interviewees in identifying what was and what was not a herbal remedy. The list of ADRs was used as a prompt if consumers were unsure what was meant by a 'side-effect', or if they answered that they had never experienced any 'side-effects' to herbal remedies. Interviewers were instructed to position themselves at an appropriate distance from the herbal remedies counter in the respective stores, and to approach customers who had purchased herbal remedies or those who had browsed the herbal remedies section. Customers were asked if they would be willing to be interviewed as part of a study on herbal remedies. Those agreeing to be interviewed were asked if they ever use herbal remedies; if they answered, 'No' the interview was terminated. If a customer answered, 'Yes', the interviewer continued with the questionnaire (written consent was not obtained); interviews took around 8 min. All questionnaires were analysed at the University of Exeter.

Two types of outlet—Boots the Chemists Ltd (BTC) and Holland & Barrett (H & B), representing a pharmacy setting and healthfood store setting, respectively—were chosen. Interviews were conducted in six BTC stores with a high turnover of herbal remedies (Manchester, Leeds, Newcastle, Milton Keynes, London, Cardiff) and in six H & B stores near the selected BTC stores (Manchester, Leeds, Newcastle, Milton Keynes, London, Swansea).

The study was conducted in September, 1996. An interviewer was present in the selected stores on 2 consecutive days for 8 h per day in BTC stores, and 4 h per day in H & B stores. The study was weighted more towards BTC customers than H & B customers to reflect market share [4].

Data collected

A structured questionnaire for customer interviews was designed and developed for this survey by researchers at the University of Exeter. Copies of the questionnaire are available on request.

Respondents were asked what herbal remedies they used, how often, and for what condition. The same questions were asked for conventional OTC medicines. Respondents were also asked if they ever used vitamins, minerals or dietary supplements, or other 'natural' health remedies (e.g. homoeopathic medicines, aromatherapy oils). Data on how respondents choose their herbal remedies and from where they obtain them, were also collected. In addition, respondents were asked if they had ever experienced any 'side-effects' after taking herbal remedies and, if so, were asked to provide the following details: name of herbal remedy; associated 'side-effect'; severity (mild, moderate or severe); if they reported the 'side-effect' and to whom; if they

stopped taking the remedy because of the 'side-effect'. Respondents were also asked for demographic information (gender, age, occupation); social grade and ethnic group were assessed by the interviewer.

The key part of the interview sought to obtain information on what action respondents would take if they experienced (a) a 'serious side-effect' (for the purposes of this survey, this was defined as symptom(s) that were 'worrying or alarming'), and (b) a 'minor side-effect' (defined as symptom(s) that 'caused some discomfort, but were not alarming') to a conventional OTC medicine and to a herbal remedy. Respondents were allowed to select one or more of the following responses: continue taking [the preparation] and see if symptom(s) resolved; stop taking immediately; consult your doctor; consult your pharmacist; consult another health care practitioner; other action.

Prior to conducting the full survey, a pilot survey was conducted. Thirty-two herbal remedy users were interviewed by one interviewer in the BTC store in Leeds. Following the pilot study, a minor alteration was made to the questionnaire (the order of the two questions on the action respondents would take following a serious and a minor ADR was reversed).

Data from the full survey were entered via a semiautomated Foxbase application into a spreadsheet for analysis. The data from the pilot study were not included in the final analysis.

Results

Six hundred and ninety individuals agreed to be interviewed. Of these, 175 (25.4%) stated that they did not use herbal remedies and therefore these interviews were terminated. Five hundred and fifteen face-to-face interviews with users of herbal remedies were conducted: 336 in BTC stores and 179 in H & B stores. Females predominated (82% overall). The ethnic origin of respondents was predominantly Caucasian (91%); Afro-Caribbean (2%), Indian/Pakistani (2%) and Chinese/Japanese (1%) ethnic groups were also represented. The age distribution of respondents was: <20 years, 2%; 20–29 years, 15%; 30–39 years, 20%; 40–49 years, 24%; 50–59 years, 19%; 60 years, 20%. There were no marked differences in age distribution between the two types of stores.

Sixty-two per cent of all respondents (58.0 and 68.2% for BTC and H & B respondents, respectively) used one or more herbal remedies *regularly*, whereas 38% (42.0 and 31.8% for BTC and H & B respondents, respectively) used one or more herbal remedies *occasionally* (respondents were allowed to name a maximum of three remedies). Eighty-one per cent of all respondents (83 and 77% for BTC and H & B respondents, respectively) were also *regular* or *occasional* users of conventional medicines; 78% (79 and 77% for BTC and H & B respondents, respectively) stated that they used vitamins, minerals and/or food supplements; 49% (51 and 45% for BTC and H & B respondents, respectively) stated that they used other 'natural' health remedies (e.g. essential oils used in aromatherapy, homoeopathic remedies).

Respondents choose their herbal remedies on the basis of a friend's or family member's recommendation (31% of replies), on the basis of their own knowledge (40% of replies)

and on the basis of a pharmacist's recommendation, on a prescription or recommendation from their doctor, and on the recommendation, or supplied by, a herbal medicine practitioner (6% of replies for each). There were no marked differences between BTC respondents and H & B respondents with regard to choosing herbal remedies except that H & B respondents were more likely than BTC respondents to choose herbal remedies recommended by a complementary health practitioner other than a herbalist (23/179 vs 11/336 for H & B vs BTC respondents, respectively; $\chi^2 = 17.37$; $P < 0.001$).

Attitudes towards reporting ADRs

Table 1 shows the numbers of respondents who would take a particular course of action after experiencing a suspected ADR to a herbal remedy and to a conventional OTC medicine. The data are presented in a manner that allows the numbers of respondents who would act differently for ADRs to herbal remedies than for similar ADRs to conventional OTC medicines to be identified.

Following a serious ADR, 156 respondents (30.3% of all replies) would consult their GP irrespective of whether the ADR was associated with the use of a herbal remedy or a conventional OTC medicine; 221 respondents (42.9%) would not consult their GP for a 'serious' ADR associated with either type of preparation. One hundred and thirty-four respondents (26.0%) would consult their GP for a serious ADR to a conventional OTC medicine, but not for a similar ADR to a herbal remedy, whereas four respondents (0.8%) would consult their GP for a serious ADR to a herbal remedy, but not for a similar ADR to a conventional OTC medicine. Similar differences were found in the attitudes of herbal-remedy users towards reporting 'minor'

ADRs associated with herbal remedies and for similar ADRs to conventional OTC medicines.

Subgroup analysis of respondents interviewed in BTC stores and those interviewed in H & B stores revealed the following differences between the two groups. Following a serious ADR, significantly more BTC respondents than H & B respondents would consult their GP irrespective of whether the ADR was associated with a conventional OTC medicine or a herbal remedy (113/336 vs 43/179 for BTC vs H & B respondents, respectively; $\chi^2 = 5.11$; $P < 0.05$). For minor ADRs, the result was reversed—H & B respondents were more likely than were BTC respondents to consult their GP irrespective of whether the ADR was associated with a conventional OTC medicine or a herbal remedy (19/179 vs 14/336 for H & B vs BTC respondents, respectively; $\chi^2 = 8.10$; $P < 0.01$). However, BTC respondents were more likely to consult a pharmacist than were H & B respondents for minor ADRs irrespective of whether the ADR was associated with a herbal remedy or a conventional OTC medicine (31/336 vs 4/179 for H & B vs BTC respondents, respectively; $\chi^2 = 9.01$; $P < 0.01$).

Subgroup analysis of 'young' respondents (<30 years of age) and 'old' respondents (>50 years) did not reveal a significant effect of age on ADR reporting for either serious or minor ADRs.

Perceptions and experience of ADRs associated with herbal remedies

Thirty-one respondents stated that they had experienced ADRs to herbal remedies. However, six of these reports referred to non-herbal complementary remedies. A further three reports cannot definitely be called herbals ('Vitalax', 'Keratine' and a product the name of which was written

Table 1 Number of respondents (% of total) that would choose a particular course of action after experiencing a suspected ADR to (i) a conventional OTC medicine and (ii) a herbal remedy. Participants responded yes or no for each type of preparation. The four groups represent respondents who answered yes for both preparations, no for both, and those who gave different answers depending on whether the ADR was associated with a conventional OTC medicine or a herbal remedy.

Nature of ADR	Likely action following ADR	Type of decision			
		Yes for both	No for both	Yes for conventional; No for herbal	No for conventional; Yes for herbal
Serious†	Continue taking and see if symptom(s) resolved	11 (2.1%)	494 (95.9%)	1 (0.2%)	9 (1.7%)
	Stop taking immediately	327 (63.5%)	108 (21.0%)	18 (3.5%)	62 (12.0%)
	Consult GP	156 (30.3%)	221 (42.9%)	134 (26.0%)	4 (0.8%)
	Consult pharmacist	34 (6.6%)	440 (85.4%)	15 (2.9%)	26 (5.0%)
Minor‡	Consult other health-care practitioner	9 (1.7%)	471 (91.4%)	0 (0%)	35 (6.8%)
	Continue taking and see if symptom(s) resolved	98 (19.0%)	374 (72.6%)	11 (2.1%)	32 (6.2%)
	Stop taking immediately	268 (52.0%)	185 (35.9%)	21 (4.1%)	41 (8.0%)
	Consult GP	33 (6.4%)	405 (78.6%)	75 (14.6%)	2 (0.4%)
	Consult pharmacist	35 (6.8%)	454 (88.2%)	10 (1.9%)	16 (3.1%)
	Consult other health-care practitioner	8 (1.6%)	482 (93.6%)	0 (0%)	25 (4.9%)

†defined as a symptom(s) that was worrying or alarming; ‡defined as a symptom(s) that gave some discomfort, but which was not alarming;
ADR=adverse drug reaction.

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illegibly). Excluding the latter three reports gives a total of 22 respondents (4.3%) who reported having experienced an ADR associated with the use of a herbal remedy. Of these, four respondents (0.8% of total) rated the adverse effect as 'severe', eight (1.6%) rated it as 'moderate', nine (1.7%) rated it as 'mild' and one entry was missing. Of the four reports rated 'severe', two were reported (both to a doctor); two were not reported. One 'severe' report ('asthma symptoms' associated with the use of Royal Jelly[†] reported to a doctor) gives cause for concern. In total, four (18%) of the 22 respondents who reported ADRs claimed to have informed their GP (we have not received replies to our letters attempting to verify these reports), 16 (73%) stopped taking the medicine concerned and six (27%) did not stop taking the preparation.

Discussion

This is the first study to provide evidence that herbal remedy users would be less likely to consult their GP for suspected ADRs (serious or minor) to herbal remedies than for similar ADRs to conventional OTC medicines. This has implications for herbal pharmacovigilance and implies that many suspected ADRs to herbal remedies will go unmonitored.

There may be several reasons for this finding. Herbal remedies are largely used on a self-treatment basis and some users may not realize that they can consult their GP about ADRs to such products. Others may be reluctant to admit herbal-remedy use to their GP by consulting him/her for suspected ADRs, while some users may feel it is more appropriate to consult the herbal practitioner from whom the remedies were obtained. In a study of unconventional medicine use involving 1539 adults in the US, Eisenberg *et al.* reported that of 34% respondents who reported using at least one unconventional therapy in the previous year, 72% did not inform their doctor of their use of the therapy [9].

There is an increasing amount of research into patients' attitudes towards complementary therapies and the reasons why people choose to use such therapies as well as, or instead, conventional medicine [10]. This is an important and complex area which is likely to have implications for ADR reporting. The present study appears to have uncovered differences between two groups of users of herbal remedies with regard to their attitudes towards reporting ADRs. BTC respondents would be more likely than H & B respondents to consult their GP for a serious ADR, irrespective of whether it was associated with a herbal remedy or a conventional OTC medicine, and would be also more likely to consult a pharmacist for a minor ADR, irrespective of whether it was associated with a herbal remedy or a conventional OTC medicine. Why there should be this difference in willingness to consult a health professional is a matter of debate. It may reflect H & B customers disenchantment with the orthodox approach to health care, or it may simply be that a health care professional is present on site in BTC stores.

[†]Although not strictly a herbal remedy, royal jelly is included here as it falls into category J (natural substances, e.g. royal jelly and herbal products) as defined by the Ministries of Agriculture Fisheries and Food (MAFF) report on Dietary Supplements and Health Foods.

Whatever the reason, the findings of the present survey raise concerns not only with regard to reporting of ADRs associated with herbal remedies, but also for those associated with conventional OTC medicines. Even for a 'serious' ADR, only 290 respondents (56.3%) would consult their GP; for 'minor' ADRs associated with conventional OTC medicines, only 108 respondents (21.0%) would do so. Also, less than 10% of respondents would consult a pharmacist for a 'serious' or 'minor' ADR associated with a conventional OTC medicine.

Even those suspected ADRs which are reported to a GP and which meet ADR-reporting criteria may not be reported on to national pharmacovigilance centres. In the UK, hospital physicians have been shown to grossly under-report ADRs that meet CSM criteria [11]; there is no evidence to suggest that GPs are any more diligent in this area. Furthermore, deficiencies in the reporting process may be even more likely to occur with herbal remedies [7]. The UK Medicines Control Agency's (MCA) and Committee on Safety of Medicine's (CSM) Yellow Card scheme already requests reports relating to suspected ADRs to all (i.e. both licensed and unlicensed) herbal remedies [12], yet (perhaps because they are not aware of this request) reporting by doctors is still limited [13]. In April 1997, the MCA extended the Yellow Card scheme to include hospital and (in certain regions) community pharmacists [13]; community pharmacists are seen as having a critical role in areas of limited reporting by doctors, e.g. over-the-counter medicines, and licensed and unlicensed herbal products [14].

There is an increasing awareness of the need to monitor the safety of herbal remedies [7]. Our findings lend support both to the MCA/CSM decision to extend its Yellow Card reporting scheme to pharmacists, and to the European Scientific Co-operative for Phytotherapy's (ESCoP) pharmacovigilance system for herbal remedies (PhytoNet). In the latter, suspected ADRs to herbal remedies may be reported via a password-protected, Internet-based reporting system which is being targeted at all health professionals who use herbal remedies.

In conclusion, consumers of herbal remedies would act differently with regard to reporting an ADR (serious or minor) to their GP, depending on whether the ADR was associated with a herbal remedy or a conventional OTC medicine. This implies that many ADRs to herbal remedies may go unmonitored. Our findings illustrate the need for greater public awareness that ADRs to herbal remedies can occur, and that such events should be reported to an appropriate authority. Professionals also need to be aware of the potential for herbal remedies to cause ADRs and routinely question their patients about their use of such remedies.

In the longer term, further research to investigate the safety and efficacy of herbal remedies is needed so that the risk/benefit ratio of using a particular herb for a specific condition can be determined.

This study was conducted as part of the European Union as part of a BIOMED (Biomedical and Health Research) programme entitled, *Determining European standards for the safe and effective use of phytomedicines*; J. Barnes holds the Boots Research Fellowship in Complementary Medicine.

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We would like to express our appreciation of the contribution to data collection of the late Mr Jeff Shaw of the market research company Benrose Shaw Berndge and Partners Ltd, Derby.

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(Received 19 September 1997,
accepted 6 January 1998)

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DEPARTMENT OF HEALTH AND HUMAN SERVICES

FD



April 27, 2004

Brent D. Rogers
Cargill Incorporated
P.O. Box 9300, MS 110
Minneapolis
MN 55440-9300

Re: GRAS Notice No. GRN 000150

Dear Mr. Rogers:

The Food and Drug Administration (FDA) has received the notice, dated April 6, 2004, that you 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)). FDA received this notice on April 9, 2004, filed it on April 13, 2004, and designated it as GRN No. 000150.

The subject of the notice is glucosamine hydrochloride. The notice informs FDA of the view of Cargill Incorporated that glucosamine hydrochloride is GRAS, through scientific procedures, for use in select beverages as defined in 21 CFR 170.3(n)(3), (7), (16), (31), (36) at a maximum level of 0.75 grams per serving.

In accordance with proposed 21 CFR 170.36(f), a copy of the information in the notice that conforms to the information described in proposed 21 CFR 170.36(c)(1) is available for public review and copying on the homepage of the Office of Food Additive Safety (on the Internet at <http://www.cfsan.fda.gov/~lrd/foodadd.html>). If you have any questions about the notice, contact me at (202) 418-3403.

Sincerely yours,

Karin Ricker, Ph.D.
Division of Biotechnology and GRAS Notice
Review
Center for Food Safety and Applied Nutrition

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Page 2 - Mr. Rogers

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000221



Ricker, Karin

From: Eshete, Feleke
Sent: Thursday, October 07, 2004 9:16 AM
To: Ricker, Karin
Subject: FW: GRAS notice 150



Questions on GRN
150.doc (38 K...

Feleke Eshete, Ph.D.
DBGNR/OFAS/CFSAN
VERB Rm. 1245
(202) 418 3241

Feshete@cfsan.fda.gov

-----Original Message-----

From: Ricker, Karin
Sent: Wednesday, June 09, 2004 1:15 PM
To: 'Brent_Rogers@cargill.com'
Cc: Martin, Robert L; Harris, Rudolph
Subject: RE: GRAS notice 150

Hello Brent,

thanks for the note.

Funny you should ask.. I was just getting ready to send you a list of questions that some of our reviewers have (please see attached file). Please let me know if you need help or clarification with any of these. Regards, Karin

-----Original Message-----

From: Brent_Rogers@cargill.com [mailto:Brent_Rogers@cargill.com]
Sent: Tuesday, June 08, 2004 4:46 PM
To: Ricker, Karin
Subject: GRAS notice 150

Hi Karin,

Time slips away quickly, and I realized it has already been a couple months since the GRAS Notification for REGENASURE glucosamine was submitted. I wanted to check to see if there were any questions I might be able to help with at this point in the review of the Notification. Thanks for assisting, I hope all is well.

Best Regards,

Brent

Brent Rogers
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Phone (641)969-3896
Cell (641)777-2467
Fax (641)696-3850

000309

GRN 150 – GLUCOSAMINE FROM CARGILL

Toxicology comments/questions

1. Cargill states that the endogenous production of glucosamine (GlucN) is in the range of 4-20 g/day (median, 14 g/day). They cite a personal communication (Hart, 2003) and three other publications on page 28 of the notice as the source of this information. These three reports they cite are about glycosylation of proteins by O-linked β -N-acetylglucosamine and its relation to mechanisms of insulin resistance, i.e. do not provide this information. In light of this, Cargill shall provide details on how they arrived at the 14 g/day figure.
2. Cargill has provided ADME information from rats, dogs and humans stating that about 90% of the ingested GlucN is absorbed, but only 20% is measured in the serum/blood. What percentage of the oral dose reaches circulation (including GlucN bound to plasma proteins and blood cells)? What happens to the 90% GlucN that reaches the liver? This has not been discussed adequately in the notice.
3. Cargill estimates an exposure of up to 98 mg/kg/day and an ADI of 184 mg/kg-bw/day. Is this level of exposure safe for diabetics given the existence of literature implicating GlucN in adverse effects in glucose homeostasis? What about in patients with rheumatoid arthritis and other inflammatory diseases of the joint?
4. Cargill describes an unpublished short-term toxicity studies (Glaza, 2002) using its GlucN product (p. 30 of the notice). Can Cargill provide a copy of the study report?
5. The table summarizing animal studies (Table 9 on page 31) is not clear in light of the absence of a detailed discussion of the studies in the notice. How were the studies designed? What endpoints were examined? What were the results and conclusions? Are the citations from published or unpublished studies? Was the table supposed to give LD₅₀ values?
6. How did the notifiers estimate the serum level of glucosamine for the oral ingestion? It appears that they have used the IV result for estimation (page 33, 1st paragraph of the notice). If so, how did they do it?
7. On page 36, Cargill discusses *in vivo* genotoxicity data from literature as well as the notifier's laboratory. How do they reconcile contradictory results from the *in vivo* genotoxicity reports? On the same subject, on page 37, Cargill states "the high dose of 2000 mg/kg selected for this (*in vivo* micronucleus assay) study was based on relevant acute toxicity information (Glaza, 2002)". Can Cargill explain

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what they mean by "relevant acute toxicity information"? Can Cargill provide us the study report on this mouse *in vivo* micronucleus assay?

8. Human clinical case reports. Disparate clinical data has been used by Cargill for meta-analysis without a discussion on how the clinical trials support the safety of GlucN in food. Which of the studies are important in supporting safety? Which clinical parameters and adverse effects data were used?
9. On transport of GluN and glucose by glucose transporters: Cargill provides this information on p. 28 and argues on p. 48 that "the concentration of GlucN in most cells will be lower than that in plasma". The work on page 28 (Uldry 2002) only states that the V_{max} values for transport of GluN by GLUT1, 2 and 4 is lower than for glucose. However, the study reports that the K_m (an indicator of the affinity that an enzyme/transporter has for a given substrate) for GlucN and glucose of GLUT1 and GLUT4 are similar, while GLUT2 (*the main transporter of GluN in hepatocytes*) has about 20-fold higher affinity for GlucN than glucose.

Chemistry questions

- 1) The notifier provides a heavy metal specification for glucosamine HCl, in accordance with USP-NF methodology. Please provide a specification for lead. (Note: analytical results for lead are provided in the notice, but there isn't a lead specification in the notice).
- 2) The notifier provides analytical results for pathogenic microorganisms. Please provide specifications for these pathogens.
- 3) The notifier states that silica is present at approximately 145 ppm in the glucosamine HCl final product (page 16 of notice). What is the source of this contaminant?
- 4) Please identify the two organic acids that were mentioned under section 3.1 (page 16 of the notice).

Microbiology question

What is the source of the organism?

000311



Meeting of Cargill and CFSAN
16 September 2003

Participants and Agenda

Participants

Brent Rogers- Cargill Acidulants
John Bohlmann- Cargill Acidulants

James W. Anderson, MD- Professor of Medicine & Clinical Nutrition- University
of Kentucky
Joseph F. Borzelleca- Professor, Pharm & Tox, Medical College of Virginia

Agenda

Introductory Comments
 Cargill and Food
 Cargill's Glucosamine- uniqueness
 Manufacturing
 Specifications

Glucosamine- Safety and Efficacy
 Efficacy
 Osteoarthritis

 Safety
 Normal biochemical pathways
 Human safety data
 Supporting safety information

GRAS status of Cargill's Glucosamine
 Scientific procedures
 Conclusions of Expert Panel (s)

000001

**Memorandum**

Date: September 16, 2003
From: Karin Ricker
Subject: Product Under Development
To: File

Participants:Visitors

Brent Rogers	Cargill Acidulants
John Bohlmann	Cargill Acidulants
Joseph Borzelleca	Medical College of Virginia
James W. Anderson	University of Kentucky

FDA


Mike DiNovi	HFS-255
Alison Edwards	HFS-255
Feleke Eshete	HFS 255
Rudolph Harris	HFS-255
Helen Lee	HFS 255
Linda Kahl	HFS-255
Antonia Mattia	HFS-255
Robert Martin	HFS-255
Charles Mize	HFS 255
Karin Ricker	HFS-255
Luis Valerio	HFS-255

The meeting was requested by Dr. Joseph Borzelleca on behalf of Cargill Acidulants to consult with FDA regarding glucosamine. The visitors discussed studies conducted with glucosamine and the intended use of the product. The visitors also discussed the evaluation of the product by an independent panel of individuals whom Cargill Acidulants considers qualified by scientific training and experience to evaluate the safety of substances added to food.

The visitors and representatives of FDA discussed the merits of data obtained from animal studies compared to data obtained from human studies. Dr. Mattia noted that FDA would be willing to continue the discussions at a second meeting.

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(b) (5)



000003

Ricker, Karin

From: Brent_Rogers@cargill.com
Sent: Monday, October 20, 2003 5:29 PM
To: Ricker, Karin
Subject: Notes from Pre-notification meeting



REGENASURE_GRA
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Dr. Ricker,

It looks to be a warm and pleasant week here, and I hope this finds the Washington, D.C. area as fortunate. I again want to express my appreciation for your coordination of the recent meeting to discuss GRAS for Cargill's REGENASURE Glucosamine.

I am attaching a very brief summary of notes, which I thought might be helpful in framing the second meeting. We continue to follow up on items from the Pre-notification meeting, and look forward to talking with you again. I would welcome any additional thoughts you or anyone in the FDA has, please pass this along to your colleagues.

Cordially,

Brent

000004


9-22-03

BDR

Cargill Confidential

**GRAS Pre-submission Conference Notes
for REGENASURE™ Glucosamine**

(b) (4)



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CONFIDENTIAL LEVEL 3 page 1



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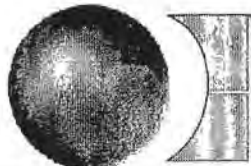
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Glucosamine HCl

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Glucosamine HCl

(b) (4)

Glucosamine Effects in Humans: Glucose Metabolism, Side Effects, Safety & Efficacy

James W. Anderson, MD
Professor of Medicine & Nutrition
University of Kentucky
Lexington, KY

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January 22, 2004

1

Introduction

- Glucosamine: endogenous amino monosaccharide component of skin, cartilage and other tissues
- Widely used for 30 years to alleviate symptoms of arthritis
- Distributed into most body tissues; kinetics, distribution similar in human, dog and rat
- Safety considerations, especially for glucose metabolism

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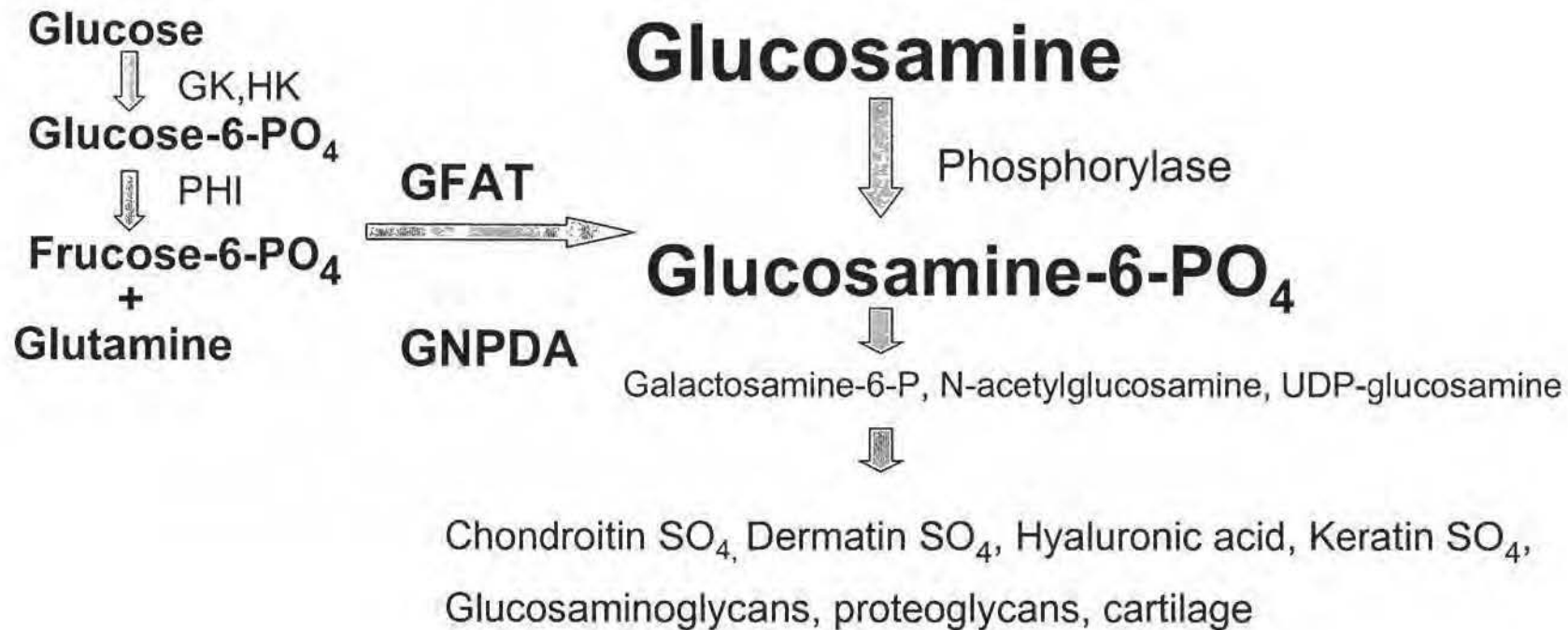
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Glucosamine Review

- Metabolism
- Effects on glucose metabolism
- Safety in humans
- Acceptable Daily Intake (ADI)
- Side effects
- Efficacy

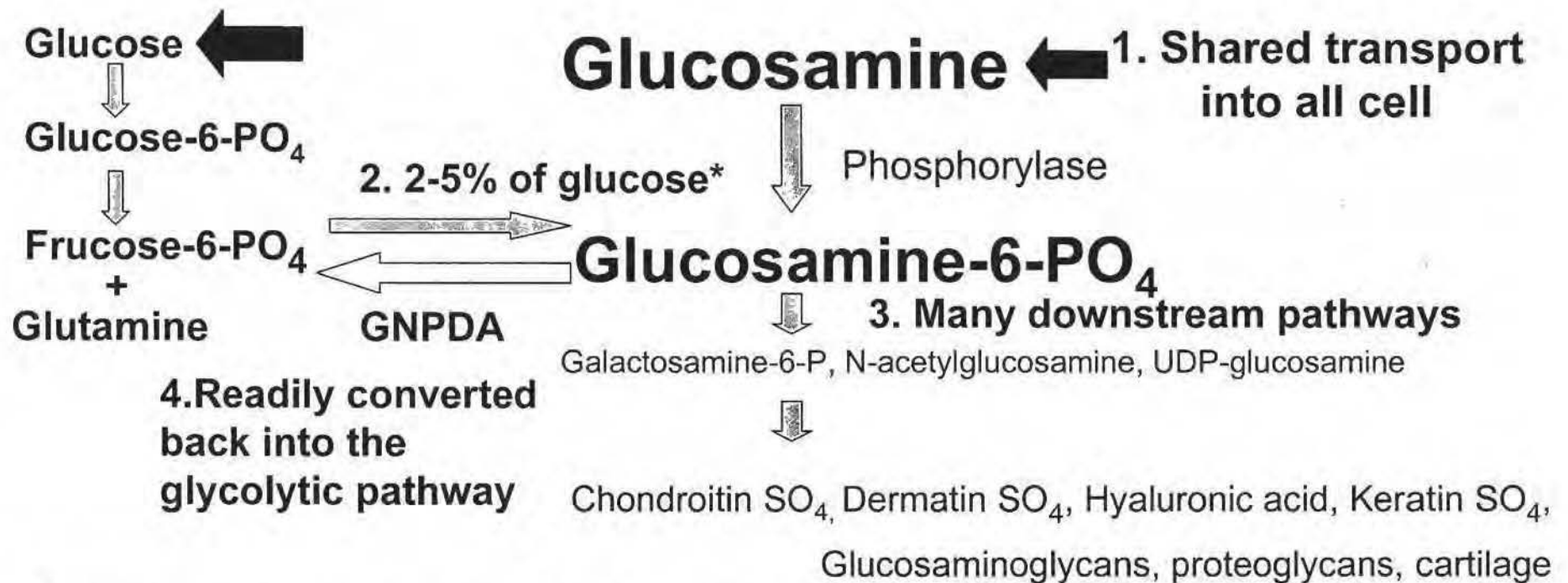
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Glucosamine Metabolism



000013 GFAT: Glutamine-F6P-amidotransferase
GNPDA: Glucosamine-6-PO₄ deaminase

Glucosamine Metabolism



*7-14 grams endogenous synthesis per day (G. Hart, JHU)

Glucosamine is transported into most tissues of the body where it is used for synthesis of proteo- and glucosamine-glycans for specific needs of the tissue. There are many downstream pathways as well as upstream conversion to F-6-P for glucose metabolism pathways₅

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Glucosamine Literature Review

- 35 human studies, 32 chronic
- 29 randomized controlled trials
- 29 used glucosamine alone, 5 incl. chondroitin SO₄
- Placebo: lactose, excipients, CaCO₃, maltodextrin, whey protein, “inert material”
- 3073 individuals; 979 pt-years

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Glucosamine Review

- Metabolism
- Effects on glucose metabolism
- Safety in humans
- Acceptable Daily Intake (ADI)
- Side effects
- Efficacy

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Effects on glucose metabolism

- Pharmacologic doses affect glucose metabolism in animals
- Doses 250-2500X human levels
- 9 studies, 336 subjects, 567 P-Y
- No effect on Fasting glucose
- ☐ 5 hr iv infus. 9 g, 20 men- no effect (Pouwels 2001; Monauni 2000)
- ☐ Conclusion: no effect at doses recommended for use

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Glucosamine Review

- Metabolism
- Effects on glucose metabolism
- **Safety in humans**
- Acceptable Daily Intake (ADI)
- Side effects
- Efficacy

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Safety in humans

- 3073 humans; 979 patient-yrs
- 21-1095 days
- **171 patient for 3 years (513 patient years)**
- 1500-3200 mg/d (20-50 mg/kg)
- No serious/life-threatening side effects
- Chem Panel, CBC, UA, occult blood, pulse, blood pressure: no sign. diff.
- No effects on glucose levels in humans
(Nondiabetic or diabetic individuals)
- Diabetes development: glucosamine, 2; placebo, 3

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Glucosamine Review

- Metabolism
- Effects on glucose metabolism
- Safety in humans
- Acceptable Daily Intake (ADI)
- Side effects
- Efficacy

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Acceptable Daily Intake (ADI)

- 9.7 g infused iv: 5 hrs, 10 men
- 9700 mg/70 kg = 138 mg/kg/day of glucosamine free base
- $138/.83^*=166$ mg/kg (Glucosamine-HCl is 83% free base)
- $166/.9^*=184$ mg/kg (GSA is 90% absorbed)
- **ADI= 184 mg/kg/day (GSA-HCl)***

***Median endogenous production = 200 mg/kg/day**

Rats & dogs tolerate 2149-2500 mg/kg/d; 0.5-1 yr

Animal toxicity studies use: 2200 X expected dose;

40 X ADI dose in mg/kg (median values)

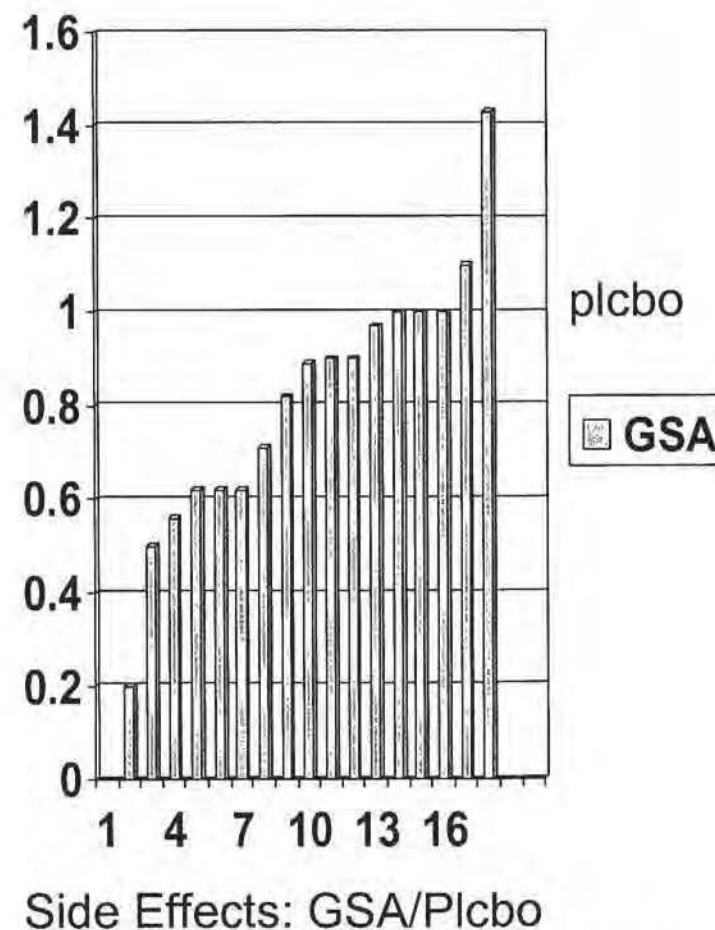
Glucosamine Review

- Metabolism
- Effects on glucose metabolism
- Safety in humans
- Acceptable Daily Intake (ADI)
- Side effects
- Efficacy

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Side Effects in Humans

- 18 studies, 906 pts
- Ratio of side effects GSA/placebo
- 14/18 less SE c GSA
- 2/18 less SE c plcbo
- Ratio 0.76 (95% CI, 0.61-0.92);
↓ 24%
- Richy meta-anal; ↓ 20%



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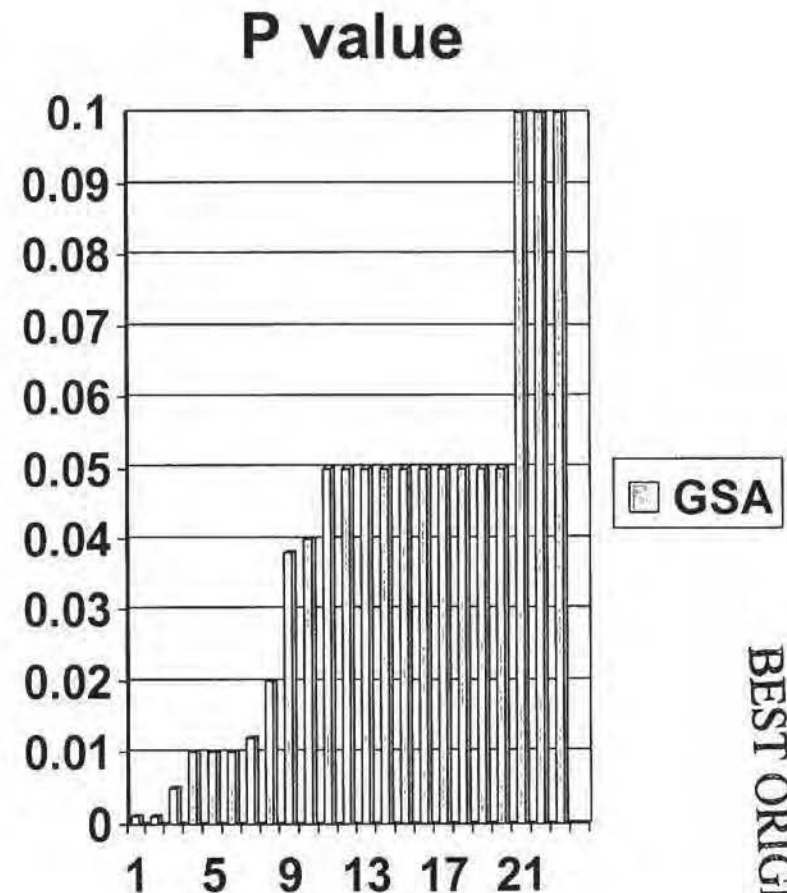
Glucosamine Review

- Metabolism
- Effects on glucose metabolism
- Safety in humans
- Acceptable Daily Intake (ADI)
- Side effects
- Efficacy

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Efficacy for Arthritis

- 23 stud, 2645 pts
- Stat sign 20/23
- Avg P= 0.04
- McAlindon meta-analy. "moderate efficacy"
- Richy meta- "Highly significant efficacy"
- **Also gets race horses back on the track sooner!**



Vs. placebo

16

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Other Consideration

- Glucosamine is endogenous
- Exogenous is well tolerated
- 90% absorbed
- Oral \Rightarrow 20% blood level of IV
- Blood level p oral ~ 0.015 mmol/L (tissue levels probably lower; glucose 5 mmol/L)
- In vitro doses 2.5-50 mmol/l (ED_{50} 25-30 mmol/l or ~ 2000 above human levels)

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From Rotta Research Laboratorium¹, Monza (Italy) and Istituto di Ricerche Biomediche "Antoine Marxer" S.p.A.²,
Ivrea-Torino (Italy)

Pharmacokinetics of Glucosamine in the Dog and in Man

By I. Setnikar¹, C. Giachetti², and G. Zanolo²

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Summary: The pharmacokinetics, organ distribution, metabolism and excretion of glucosamine were studied in the dog giving uniformly labelled [¹⁴C]-glucosamine (sulfate), i.v. or orally, in single doses.

Immediately after i.v. administration, the radioactivity in plasma is due to glucosamine, and freely diffuses into organs and tissues. This radioactivity disappears quickly from plasma (initial $t_{1/2}$ = 13 min, terminal $t_{1/2}$ = 118 min). After 30–60 min the radioactivity in plasma is no longer due to glucosamine, but is incorporated into α - and β -globulins. The protein-incorporated radioactivity is found already 20–30 min after i.v. administration, reaches a peak after 8 h and then slowly disappears, with a $t_{1/2}$ = 2.9 days. Of the administered radioactivity, more than 34% is excreted in the urine, mainly as glucosamine, and 1.7% is excreted in the feces. Radioactivity is excreted also as [¹⁴C]-CO₂ in the expired air. The radioactivity, after i.v. administration, diffuses rapidly from blood into the body. Some organs show an active uptake of radioactivity, e.g. the liver and the kidney. Other tissues, such as the articular cartilage, also have an active uptake. In most other organs the radioactivity found can be explained by passive diffusion processes from plasma. After oral administration of a single dose of [¹⁴C]-glucosamine the radioactivity is quickly and almost completely absorbed from the gastrointestinal tract. The pattern of disappearance, metabolic transformation, tissue distribution and excretion of the radioactivity are consistent with those found after i.v. administration.

In man unlabelled glucosamine sulfate (Dona® 200-S) was given i.v. and orally and glucosamine was measured in plasma and urine with a glucosamine-specific ion-exchange chromatographic method. The results show that the bioavailability, pharmacokinetics and excretion pattern of glucosamine are consistent with those found in the dog with radio-labelled glucosamine, and with those reported in a previous study in the rat.

Zusammenfassung: Pharmakokinetik von Glucosamin beim Hund und beim Menschen

Die Pharmakokinetik, Verteilung in den Organen, der Metabolismus und die Ausscheidung von Glucosamin wurden

am Hund nach einmaliger intravenöser oder oraler Applikation von uniform markiertem [¹⁴C]-Glucosaminsulfat untersucht. Unmittelbar nach der intravenösen Applikation verschwindet die Glucosamin-Radioaktivität rasch aus dem Plasma (initiale Halbwertszeit = 13 min, terminale Halbwertszeit = 118 min). Nach 30–60 min ist keine Radioaktivität durch freies Glucosamin mehr vorhanden, sondern nur noch Radioaktivität von Glucosamin-Derivaten, die in α - und β -Globuline inkorporiert wurden. Die in Proteine inkorporierte Radioaktivität wird schon 20–30 min nach intravenöser Applikation gefunden, erreicht ihren Höhepunkt nach 8 h und sinkt dann langsam ab, mit einer Halbwertszeit von 2,9 Tagen. Mehr als 34% der applizierten Radioaktivität werden mit dem Urin, vorwiegend als Glucosamin, ausgeschieden. Nur 1,7% werden mit den Fäzes ausgeschieden. Radioaktivität wird auch mit der Atemluft als [¹⁴C]-CO₂ ausgeschieden. Die Radioaktivität nach intravenöser Applikation verteilt sich schnell vom Blut in den Organismus. Manche Organe nehmen die Radioaktivität stark auf, insbesondere die Leber und die Nieren. Auch andere Gewebe, wie der Gelenkknorpel, nehmen die Radioaktivität auf. In den meisten Organen ist die dort gefundene Radioaktivität auf passive Diffusionsprozesse aus dem Plasma zurückzuführen. Nach oraler Applikation einer Einzeldosis von [¹⁴C]-Glucosamin beim Hund wird die Radioaktivität schnell und vollständig aus dem Gastrointestinaltrakt resorbiert. Die Plasmaelimination, der Metabolismus, die Verteilung im Gewebe und die Ausscheidung der Radioaktivität entsprechen der nach intravenöser Applikation.

Die Untersuchungen am Menschen wurden mit nicht-markiertem Glucosaminsulfat (Dona® 200-S), das intravenös oder oral gegeben wurde, durchgeführt. Glucosamin wurde mit Hilfe der Ionentauscher-Chromatographie nachgewiesen, einer Glucosamin-spezifischen Methode, die jedoch nur relativ hohe Glucosamin-Konzentrationen und keine Glucosamin-Metaboliten erfaßt. Trotz dieser Einschränkungen stimmen die mit nicht-markiertem Glucosamin beim Menschen gefundenen Ergebnisse bezüglich der Bioverfügbarkeit, Pharmakokinetik und Ausscheidung mit denen überein, die beim Hund mit radioaktiv markiertem Glucosamin gefunden wurden. Ebenso stehen die Resultate mit früher berichteten Erkenntnissen bei der Ratte im Einklang.

Key words: Antirheumatics · Dona® 200-S · Glucosamine, clinical studies, pharmacokinetics

1. Introduction

Glucosamine is an amino sugar biosynthesized from glucose and used by the body as a molecular element for special macromolecules, the proteoglycans, important constituents of the articular cartilage. Certain articular disorders, e.g. osteoarthritis, are due to a "degenerative" process affecting the cartilage. The causes of this "degenerative" process are

not known. Surely, however, an important factor is represented by a defect of the biosynthesis and the turnover of glucosamine and of other aminosugars [5]. In fact, the supply of exogenous glucosamine in form of sulfate is able to influence favourably the osteo-arthritic process and is currently used for therapeutic purposes [2, 3, 8, 14, 15].

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Setnikar et al. - Glucosamine

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Exogenous glucosamine can therefore be considered as a pharmacological agent and the knowledge of its pharmacokinetics and drug metabolism is important in order to rationalize its therapeutic use.

The pharmacokinetics of exogenous glucosamine was already studied in the rat [11], using uniformly labelled [^{14}C]-glucosamine (sulfate).

In the present study the results found in dog and man are given.

2. Materials and methods

2.1. Animals

Beagle dogs supplied by G. Soprani, S. Paolo d'Enza (Italy) were used. The animals were kept in individual wire cages, and the urine and feces quantitatively collected. Temperature was $24 \pm 2^\circ\text{C}$, humidity $60 \pm 20\%$, with two 12-h photo periods per day. Acclimatization lasted at least 2 weeks. The animals were fed with 6TH30 pellets from Ditta Italiana Mangimi, Settimo Milanese (Italy). During some experiments the animals were kept in metabolic cages, to collect also the expired CO_2 .

2.2. Volunteers

Healthy volunteers of both sexes were informed about the study and gave their consent. Glucosamine sulfate was administered in dosage forms and dosages authorized for therapeutic use.

2.3. Materials

Uniformly labelled [^{14}C]-glucosamine was obtained from Amersham International Limited (Amersham, UK), with a specific activity of 1.23 mCi/mg. The product is supplied as hydrochloride in a 0.615% solution in water. Before administration, the product was diluted with unlabelled glucosamine sulfate and NaCl to obtain the desired specific activity. The solution to be administered had the stoichiometric composition of glucosamine sulfate for therapeutic use. The doses are referred to glucosamine sulfate.

Plasma was obtained centrifuging heparinized venous blood.

2.4. Procedures

2.4.1. Determination of the pK of glucosamine

Aqueous solutions of glucosamine hydrochloride, at different concentrations, were titrated with NaOH using the E576 Potentiograph, the Dosimet E535 and the glass combined electrode EA121 of Metrohm Ltd., Herisau (Switzerland). The pH of the half-titration were extrapolated to zero concentration of glucosamine, to determine the pK_a .

2.4.2. In vitro plasma protein binding by equilibrium dialysis

Radioactive glucosamine was conveniently diluted with unlabelled glucosamine sulfate and added to human, dog or rat plasma, in dialysis cells of 1 ml for the plasma compartment and 1 ml for the protein-free compartment. The dialysis was performed for 6 h at 0°C , without agitation. Used were No. 3687 F 75 M.W. Cut-off 12 000 membranes, supplied by Arthur H. Thomas Co., Philadelphia, PA (USA). The final dilutions of glucosamine in plasma were 0, 3, 10 and 30 $\mu\text{g/ml}$ (0, 0.02, 0.07 and 0.21 $\mu\text{Ci/ml}$). The radioactivity was counted as described below for plasma.

2.4.3. Electrophoresis

A Gelman ACD 18 automatic apparatus was used, on cellulose acetate Sepharose III Gelman support (Gelman Instrument Co., Ann Arbor, MI, USA). Ponceau-S was used for staining. Deposited were 5–7 μl of plasma, and run at 200 V for 20 min. Reading was performed with the Sepratek Gelman method.

Autoradiographies were obtained exposing the electrophotograms by contact to photographic films (No-Screen Film, Kodak) at -20°C for 3–10 weeks, as appropriate. The film was then developed by normal photographic procedures.

2.4.4. Measurement of radioactivity

An Intertechnique SL 32 counter (Plaisir, France) was used. The dpm values were calculated using an external channel ratio method, with reference to a previously prepared standard quenching curve. Instagel, Dimilume 30, Carbosorb, Soluene 350 were all obtained from Packard Instruments (Milano, Italy). In plasma the total radioactivity was counted directly in Instagel. A portion of plasma was deproteinized with an equal volume of 10% trichloroacetic acid and centrifuged. The supernatant was counted directly in Instagel.

Red blood cells were digested in Soluene 350 at 40°C in a shaking Dubnoff bath, bleached with a mixture of hydrogen peroxide (30%) and isopropanol, and finally counted in Dimilume 30. In urine the radioactivity was counted directly in Instagel. The feces were dried at 70°C under vacuum for one night and homogenized. Weighed aliquots were rehydrated, digested, bleached and counted as described for red cells.

Tissues and organs were minced with scissors. Weighed aliquots of each tissue were digested, bleached and counted as described for red cells.

2.4.5. Assay of glucosamine by ion-exchange chromatography

Plasma (1 ml) was deproteinized with 30 mg of sulfosalicylic acid and centrifuged at 4000 rpm for 5 min. Aliquots of 0.2–0.4 ml of the supernatant were analyzed with the ion-exchange chromatographic method [6, 7]. Urine was diluted 1:4 with lithium buffer pH 2.8, filtered and aliquots of 1 ml were used. The chromatographic separation was made in an Optica Aminoacid Analyzer (Optica, Milan, Italy), in a glass column of 45 cm length and 11.4 cm i.d., packed with Amberlite CG 120 resin, 32 cm in height. Lithium citrate buffer was used as carrier, at a flow rate of 1.4 ml/min, 190 ml pH 2.8, then pH 4.15. The starting temperature was 34°C , after 86 min it was increased to 64°C . Glucosamine was assayed after reaction with a ninhydrin solution flowing at 0.7 ml/min in a boiling water bath, in a colorimeter equipped with a 570 and 440 nm interference filters and a cylindrical flow cuvette with 10 mm light path. The composition of the ninhydrin solution was: ninhydrin 20 g, hydridant in (cf. [6]) 0.8 g, 2-methoxyethanol 740 ml, acetate buffer 4 mol/l pH 5.5 260 ml. In these conditions glucosamine has a retention time of 125–130 min and peaks in a clean region of the chromatogram. The minimum detectable amount is 5–15 nmol/l of glucosamine (1–3 $\mu\text{g/ml}$).

2.5. Pharmacokinetics of radioactivity in dogs after i.v. administration of uniformly labelled [^{14}C]-glucosamine sulfate

A quantity of 250 μCi of [^{14}C]-glucosamine sulfate was diluted with cold glucosamine sulfate to inject i.v. 10 mg/kg of glucosamine sulfate. Blood was sampled after 5, 10, 15, 20, 30, 45, 60, 90 and 120 min in a group of 2 male and 2 female dogs, and also after 4, 8, 24, 48, 72, 96 and 144 h from a second group of 2 male and 2 female dogs. Radioactivity was measured in a portion of plasma and also on the supernatant of an other portion, which was deproteinized with 10% trichloroacetic acid. The precipitable radioactivity was calculated by difference.

Urine was sampled only in the second group of dogs, between 0 and 8, 8 and 24, 24 and 48, 72 and 96, and 96 and 144 h after administration. In these dogs the feces were collected at the end of each 24-h period after administration. The first group was sacrificed 2 h after administration and the organs collected for measuring the radioactivity. The second group was sacrificed after 144 h to measure the radioactivity in the organs. The characteristics of the dogs are given in Table 1.

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Table 1: Characteristics of dogs.

	Administration	
	I.v.	Oral
No. of dogs	8 (4 σ , 4 ϕ)	8 (4 σ , 4 ϕ)
Average weight (kg)	10.84	10.19
S.D.	0.50	1.59
Range	10.2–11.6	8.1–12.3

2.6. Pharmacokinetics of radioactivity in dogs after oral administration of uniformly labelled [^{14}C]-glucosamine sulfate

Cold glucosamine sulfate was added to 250 μCi of [^{14}C]-glucosamine to administer by gastric tube 10 mg/kg of glucosamine sulfate. Blood was sampled after 15, 30, 45, 60, 75, 90, 120, 150, 180 and 240 min in a group of 2 male and 2 female dogs, and also after 4, 8, 24, 48, 72, 96 and 144 h in a second group of 2 male and 2 female dogs. Measured were the total radioactivity in plasma and the radioactivity in the supernatant after precipitation of the plasma proteins with 10% trichloroacetic acid. The radioactivity in plasma proteins was calculated by difference. Urine was sampled in the second group of animals, between 0 and 8, 8 and 24, 24 and 48, 48 and 72, 72 and 96, 96 and 120, 120 and 144 h. Feces were sampled in the second group of dogs at the end of each 24-h period. The first group of dogs was sacrificed 4 h after administration, the second group 144 h after administration, and the radioactivity in the organs was determined. The body weight of the animals is given in Table 1.

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2.7. Pharmacokinetics of glucosamine in human volunteers after i.v. administration

Glucosamine sulfate* was injected in the cubital vein of 6 volunteers, whose characteristics are given in Table 2. Blood was sampled 10, 20, 30, 45, 60, 90, 120, 180 and 240 min after administration, the plasma was separated and was assayed for glucosamine with the ion-exchange chromatographic method. The urines were collected between 0 and 2, 2 and 8, and 8 and 24 h after administration, and assayed for glucosamine with the ion-exchange chromatographic method.

2.8. Pharmacokinetics of glucosamine in human volunteers after oral administration

Glucosamine sulfate was given in commercial sugar-coated tablets* to 6 volunteers, whose characteristics are shown in Table 2. Blood was sampled 15, 30, 45, 60, 90, 120 and 180 min after administration. Urine was collected between 0 and 4, 4 and 8, 12 and 24 h after administration.

Table 2: Characteristics of human volunteers (average \pm S.D.).

	I.v.	Oral
Number	6 (3 ♂, 3 ♀)	6 (3 ♂, 3 ♀)
Age	34.8 \pm 10.9	29.3 \pm 6.9
Range	20 – 48	24 – 42
Weight	58.5 \pm 10.7	55.2 \pm 8.5
Range	47 – 75	42 – 67
Height	169.7 \pm 8.5	169.2 \pm 10.2
Range	163 – 184	150 – 180

3. Results

3.1. pK of glucosamine

The pK_a of glucosamine was found equal to 7.52 at 20 °C, and 6.91 at 37 °C.

3.2. Binding to plasma proteins in vitro

Concentrations of glucosamine sulfate between 3.82 and 38.7 μ g/ml in human, canine or rat (Sprague-Dawley) plasma, freely diffused through the dialyzing membrane. In vitro, therefore, glucosamine is not bound to plasma proteins of man, dog or rat.

3.3. Pharmacokinetics of radioactivity in dogs, after i.v. administration of uniformly labelled [¹⁴C]-glucosamine sulfate

The radioactivity found in plasma, splitted into non-precipitable and precipitable radioactivity, is given in Fig. 1.

3.3.1. Total radioactivity in plasma

The total radioactivity declines rapidly, till the 54th min after injection. Then it increases and 8 h after injection it reaches the same levels as 5 min after injection. Afterwards it decreases very slowly (disappearance $t_{1/2}$ = 70 h). This unusual pharmacokinetic behavior is similar to that found in the rat [11].

3.3.2. Radioactivity in the non-precipitable fraction

The percentage of radioactivity in the non-precipitable fraction with regard to the total radioactivity is given in Table 3. Until 30 min after injection, 90% of the radioactivity or more is in the non-precipitable fraction. Then this radioactivity decreases rapidly. After 90 min it represents only 19% of the total radioactivity. After 4 h it is less than 5%, and is probably an artifact due to incomplete precipitation. Samples of the non-precipitable fraction were assayed by ion-exchange chromatography. The retention time of the radioactivity was the same as that of glucosamine. Also the amount of radioactivity was consistent with the concentrations of glucosamine found by the ion-exchange chromatographic method.

The radioactivity of the non-precipitable fraction until 60 min after injection represents therefore free glucosamine in

* Dona® 200-S; manufacturer: Opfermann Arzneimittel GmbH, Bergisch Gladbach (Federal Republic of Germany).

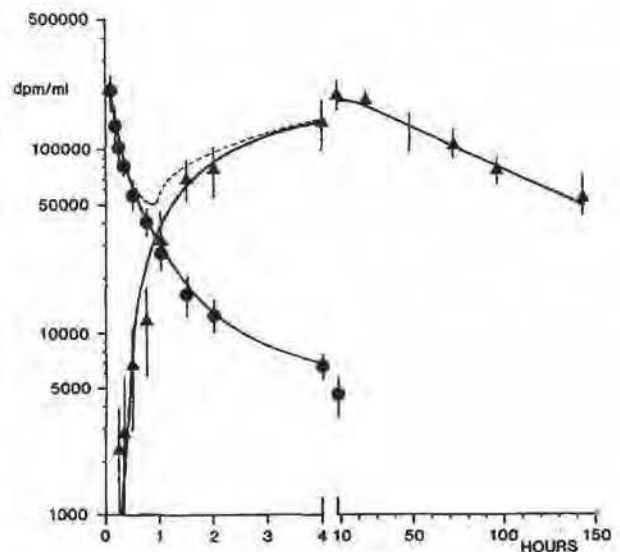


Fig. 1: Radioactivity (---, total), in deproteinized plasma (●—●) (mainly due to glucosamine) and in plasma proteins (▲—▲) (due to glucosamine fractions incorporated in α - and β -globulins) after i.v. injection of 23 μ Ci/kg of [¹⁴C]-glucosamine (10 mg/kg glucosamine sulfate) to dogs. Averages and standard deviations are given.

Table 3: Radioactivity in the non-precipitable fraction of plasma, in percent of total plasma radioactivity.

Time (h)	Radioactivity (%) after	
	I.v. route	Oral route
0.083	97	
0.17	99	
0.25	98	
0.33	97	98
0.50	90	96
0.75	77	76
1.00	45	82
1.25		66
1.50	19	53
2.00	14	38
2.50		24
3.00		22
4.00	4	13
Afterwards	5	6

plasma. After 60 min the levels of free glucosamine were too small to be studied with the ion-exchange method.

Two electrophoretograms were obtained from each plasma sample. One was stained with the usual method to measure albumins and globulins. The other was autoradiographed. Till 10–20 min after injection the radioactivity remained at the starting spot. Between 30 and 60 min the radioactivity in the electrophoretogram was small and not clearly concentrated at any level. After 60 min the precipitable radioactivity was concentrated in few bands, which were associated with the α - and β -globulins.

3.3.3. Pattern of the non-precipitable radioactivity

Using the stripping method [16], the following exponential equation (1) was found for the average levels of non-precipitable radioactivity:

$$1000 \text{ dpm/ml} = 192 \times e^{-3.208 \times h} + 26.7 \times e^{-0.352 \times h} \quad (1)$$

Since the administered radioactivity was 23 μ Ci/kg of body weight, the initial distribution volume can be calculated as 105 ml/kg body weight. The initial disappearance rate has a $t_{1/2}$ of 13 min. It represents probably the diffusion of glucosamine from the vascular bed. A terminal disappearance rate follows with a $t_{1/2}$ of 1.97 h. It represents probably the diffusion of glucosamine into the tissues and the uptake of glucosamine for biosynthesis and metabolic processes. The levels after the 8th h were excluded from the analysis because affected by a large experimental error.

3.3.4. Pattern of the precipitable radioactivity

The first precipitable radioactivity appears ca. 15 min after i.v. administration and then increases rapidly. The radioactivity pattern is described by equation (2):

$$1000 \text{ dpm/ml} = -229 \times e^{-0.317 \times h} + 214 \times e^{-0.010 \times h} \quad (2)$$

In equation (2) the first term represents the "invasion" phase and describes probably the kinetics of the biosynthetic process of plasma proteins in which glucosamine is involved. The peak occurs 12 h after administration, and is 185×10^3 dpm/ml (calculated). Then the precipitable radioactivity decreases, with a $t_{1/2}$ of 69.3 h. The distribution volume, calculated by extrapolation of the disappearance curve to time 0, is 107 ml/kg body weight, i.e. very similar to the initial distribution volume of the radioactivity due to glucosamine.

The AUC, calculated by the trapezoid method between 15 min and 144 h, is 15.8×10^6 dpm/ml \times h.

3.3.5. Radioactivity in red cells

The radioactivity found in red cells represents the sum of glucosamine entered into the erythrocytes, the radioactivity bound to the membrane of the erythrocytes and the contamination by plasma radioactivity. Due to this composite origin, the radioactivity is difficult to analyze, also because it is affected by a notable quenching error. In general the radioactivity in the erythrocytes was proportional to the non-precipitable radioactivity in plasma.

3.3.5. Urinary and fecal excretion of radioactivity

Table 4 gives the excretions of radioactivity found in urine and feces. During 6 days of observation 35.9% of the administered radioactivity was excreted with the urine and feces. Fecal radioactivity was 5% of the urinary radioactivity.

Table 4: Cumulated radioactivity found in urine and feces after i.v. administration of [14 C]-glucosamine. Average \pm S.D. of the percent of administered dose calculated on the results from 4 animals.

Time (h)	Urine	Feces	Total
8	18.0 \pm 9.9		18.0 \pm 9.9
24	29.6 \pm 3.3	0.88 \pm 0.13	30.5 \pm 3.4
48	31.9 \pm 3.3	1.42 \pm 0.14	33.3 \pm 3.4
72	32.9 \pm 3.7	1.58 \pm 0.16	34.5 \pm 3.8
96	33.7 \pm 3.6	1.66 \pm 0.19	35.3 \pm 3.7
144	34.2 \pm 3.5	1.71 \pm 0.20	35.9 \pm 3.6

Table 5: Radioactivity found in organs after i.v. administration of 23 μ Ci/kg b.w. of [14 C]-glucosamine.

Organ	After 2 h		After 144 h	
	1000 dpm/g	% ^a	1000 dpm/g	% ^a
Liver	861	44.8	67	4.3
Kidney	273	26.1	107	1.2
Adrenals	36	0.010	57	0.02
Brain	111	0.93	36	0.46
Heart	17	0.30	12	0.21
Lungs	43	1.10	22	0.49
Uterus	135	0.14	40	0.04
Ovaries	325	0.04	87	0.01
Testes	18	0.076	25	0.12
Cartilage ^b	170		17	
Muscle	70		7	
Spleen	270	0.43	27	0.17
Sternum	24	0.20	29	0.07
Pancreas	14	0.60	17	0.52
Bone marrow	29		21	
Eye	5	0.012	2	0.006
Subcutaneous fat	9		11	
Perirenal fat	12		24	
Skin	13		12	
Glandular stomach	19	0.079	19	0.074
Mucosal stomach	12	0.17	22	0.44
Small intestine	52		23	1.73
Plasma (non-precip.)	13		1	

^a Percent of administered dose found in the whole organ. ^b From the femoral head.

Most of the urinary radioactivity (87%) was excreted in the first 24 h. By ion-exchange chromatography it was found that the radioactivity in the urine of the first 24 h was due to glucosamine by ca. 80%.

3.3.6. Radioactivity in the expired air

In the rat study [11] substantial amounts of radioactivity were eliminated as CO₂ in the expired air. The presence of [14 C]-CO₂ in the expired air was found also in the dogs after i.v. administration of radioactive glucosamine. Our experimental conditions, however, did not allow to make a quantitative collection of the expired CO₂.

3.3.7. Radioactivity in organs

The radioactivity found in organs and tissues, 2 and 144 h after administration, is given in Table 5. 2 h after administration the greatest radioactivity is found in the liver and after 144 h in the kidneys. Notable is the radioactivity found in the cartilage, which is greater than that found e.g. in other tissues, such as the skeletal muscles. Even 144 h after administration there is a notable residual radioactivity in most organs and in the cartilage. These results are consistent with those found in a previous study in the rat [11].

3.4. Pharmacokinetics of radioactivity, after oral administration of [14 C]-glucosamine sulfate

The radioactivity found in plasma, splitted into non-precipitable and precipitable radioactivity, is given in Fig. 2.

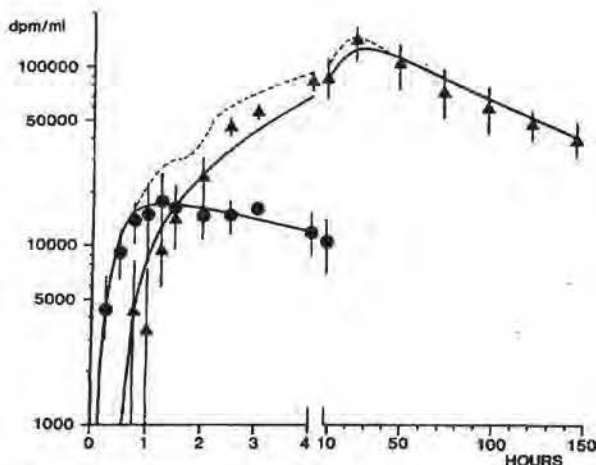


Fig. 2: Radioactivity (---, total), in deproteinized plasma (●—●) (mainly due to glucosamine) and in plasma proteins (▲—▲) (due to glucosamine fractions incorporated in alpha and beta globulins) after oral administration of 24.5 μ Ci/kg of [14 C]-glucosamine (10 mg/kg glucosamine sulfate) to dogs. Average and standard deviations are given.

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3.4.1. Total radioactivity in plasma

Radioactivity appears already 15 min after oral administration. It increases to reach the peak level 24 h after administration. Afterwards it decreases slowly with a linear disappearance rate characterized by a $t_{1/2}$ of about 60 h.

The total radioactivity can be separated into a fraction which precipitates with the plasma proteins and a fraction which does not precipitate with the plasma proteins.

3.4.2. Radioactivity in the non-precipitable fraction

Until 60 min after administration, most of the radioactivity is in the non-precipitable fraction of plasma. It reaches the peak at 60 min after administration and then it declines slowly. The non-precipitable radioactivity represents 98% of the total radioactivity 15 min after administration, then the percentage decreases to 82% after 1 h, 38% after 2 h and is less than 6% of the total radioactivity after 4 h (Table

Table 7: Radioactivity found in organs after oral administration of 25 µCi/kg b.w. of [¹⁴C]-glucosamine.

Organ	After 2 h		After 144 h	
	1000 dpm/g	%a)	1000 dpm/g	%a)
Liver	467	19.3	60	2.7
Kidney	137	1.1	76	0.76
Adrenals	31	0.05	48	0.011
Brain	31	0.45	15	0.20
Heart	13	0.20	12	0.23
Lungs	25	0.36	22	0.40
Uterus	25	0.045	31	0.009
Ovaries	38	0.008	40	0.071
Testes	10	0.04	18	0.080
Cartilage ^{b)}	12		8	
Muscle	7		8	
Spleen	27	0.11	49	0.22
Sternum	14	0.040	16	0.058
Pancreas	4	0.076	10	0.23
Bone marrow	21		14	
Eye	11	0.02	10	0.024
Subcutaneous fat	5		9	
Perirenal fat	10		12	
Skin	5		9	
Glandular stomach	14	0.056	16	0.056
Mucosal stomach	11	0.17	15	0.24
Small intestine	68	4.4	18	1.1
Plasma (non-precip.)	12		3	

a) Percent of administered dose found in the whole organ. b) From the femoral head.

the residual radioactivity is larger than the non-precipitable radioactivity in plasma, showing that a part of the radioactivity is represented by glucosamine or glucosamine derivatives incorporated into the components of the tissues.

3.5. Plasma levels and urinary excretion after i.v. administration in man of 800 mg glucosamine sulfate

The plasma levels of glucosamine are given in Fig. 3. The results, analyzed with the stripping method [16], can be interpreted by equation (5) for a two-compartment open model:

$$C = 119.8 \times e^{-6.77 \times h} + 20.7 \times e^{-0.336 \times h} \quad (5)$$

where C is the concentration of glucosamine in ng/ml. The pharmacokinetic constants are as follows: distribution volume = 71 ml/kg; first disappearance rate $t_{1/2} = 6.1$ min; terminal disappearance rate $t_{1/2} = 2.1$ h. Equation (5) shows that after i.v. administration, the pharmacokinetics in plasma of glucosamine in man is comparable to that of the non-precipitable fraction of radioactivity in dogs (cf. equation (1)).

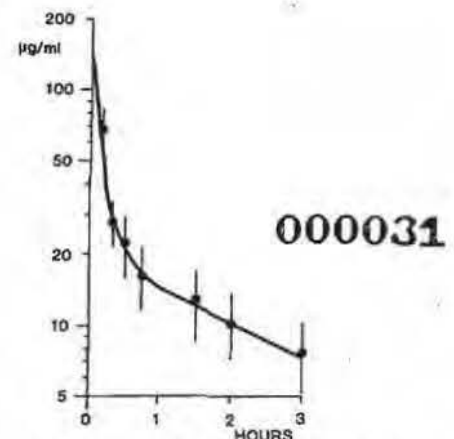


Fig. 3: Plasma levels of glucosamine in 6 volunteers after i.v. administration of 13.7 mg/kg glucosamine sulfate. The assay was made with ion-exchange chromatography, a method which is specific for glucosamine. Levels are expressed in glucosamine base. Averages and standard deviations are given.

3). This small quantities after the 4th h are probably artifacts due to incomplete protein precipitation. By ion-exchange chromatography it was seen that most of the non-precipitable radioactivity is due to unchanged glucosamine. The average plasma levels of the non precipitable radioactivity were analyzed with the stripping method [16] and fitted with a single-compartment open model (equation 3):

$$1000 \text{ dpm/ml} = -34.2 \times e^{-2.826 \times h} + 22.1 \times e^{-0.150 \times h} \quad (3)$$

The invasion rate is very fast and has a $t_{1/2}$ of 15 min. The disappearance rate is defined by a $t_{1/2}$ of 4.62 h. The disappearance rate of the non-precipitable radioactivity after oral administration is probably due to the same factors as that after i.v. administration.

3.4.3. Radioactivity in the precipitable fraction

In the precipitable fraction of plasma, radioactivity appears in appreciable amounts 1 h after administration. It increases rapidly and reaches the peak 30 h after administration with 128.8×10^3 dpm/ml. Afterwards it declines slowly with a disappearance $t_{1/2}$ of 63 h. The pattern of the precipitable radioactivity can be described by equation (4):

$$1000 \text{ dpm/ml} = -189.8 \times e^{-0.111 \times h} + 180.9 \times e^{-0.011 \times h} \quad (4)$$

The "invasion" phase is characterized by a $t_{1/2}$ of 6.2 h (after i.v. administration the "invasion" $t_{1/2}$ is 2.2 h). The AUC between 30 min and 144 h is 11.3×10^6 dpm/ml \times h. The ratio between this AUC and that after i.v. administration is a good index of the bioavailability of glucosamine sulfate orally given. This ratio is 0.715.

3.4.4. Radioactivity in red cells

The radioactivity in red cells is due to the radioactivity of glucosamine, to the radioactive substances bound to the membrane, and to contamination with plasma. Due to this composite origin, this radioactivity is difficult to analyze. However, it can be explained by the non-precipitable radioactivity freely diffusing from plasma into the red cells.

3.4.5. Urinary and fecal excretions of radioactivity

Table 6 gives the cumulated urinary and fecal excretion of radioactivity.

In 144 h 22.3% of the administered radioactivity is excreted via the urinary and fecal route. The radioactivity excreted with feces is 12.7% of the administered radioactivity. This means that at least 87.3% of the administered glucosamine was absorbed from the gastrointestinal tract.

Table 6: Cumulated radioactivity found in urine and feces after oral administration of [¹⁴C]-glucosamine. Average \pm S.D. of the percent of administered dose calculated on the results from 4 animals.

Time (h)	Urine	Feces	Total
8	4.7 \pm 0.8		4.7 \pm 0.8
24	7.7 \pm 1.7	2.4 \pm 1.6	10.1 \pm 3.2
48	8.5 \pm 1.4	10.7 \pm 5.4	19.2 \pm 4.1
72	9.1 \pm 1.3	11.9 \pm 5.6	21.0 \pm 4.5
96	9.3 \pm 1.3	12.3 \pm 5.5	21.7 \pm 4.5
120	9.5 \pm 1.3	12.6 \pm 5.5	22.1 \pm 4.4
144	9.6 \pm 1.3	12.7 \pm 5.5	22.3 \pm 4.4

3.4.6. Radioactivity in the expired air

In samples of expired air [¹⁴C]-CO₂ was found. It was not possible to measure it quantitatively.

3.4.7. Radioactivity in organs

The radioactivity found in organs and in tissues, 4 or 144 h after administration, is given in Table 7. The distribution of radioactivity is consistent with that found in the rat [11]. 4 h after administration the greatest radioactivity is found in the liver, followed by the kidneys. Other organs and tissues, including the articular cartilage, show an active uptake of radioactivity from plasma. Even 144 h after administration there is a notable residue of radioactivity. In most organs

The urinary excretions are given in Table 8. The excretion occurs mainly within the first 2 h after administration. A smaller fraction is excreted in the following 6 h, reaching a total of 38.3% of the administered dose. It should be recalled that in dogs the urinary excretion of radioactivity 24 h after i.v. injection was $29.6 \pm 3.3\%$ of the administered dose (cf. Table 4). The results obtained in man are therefore similar to those obtained in the dog.

Table 8: Urinary excretions after i.v. administration in man of 800 mg of glucosamine sulfate in percent of administered dose. Average \pm S.D. of 6 subjects.

Time (h)	Fraction	Cumulated
0-2	31.7 ± 5.1	31.7 ± 5.1
2-8	6.6 ± 1.2	38.3 ± 5.6
8-24	0	38.3 ± 5.6

3.6. Plasma levels and urinary excretions after oral administration in man of 6 grams of glucosamine sulfate

In plasma glucosamine was below the detection limit (ca. 10 $\mu\text{g/ml}$), at all tested times. Conversely in urine substantial amounts of glucosamine could be found, as shown in Table 9. Glucosamine is therefore absorbed from the gastrointestinal tract.

Table 9: Urinary excretions after oral administration in man of 6 grams of glucosamine sulfate, in percent of the administered dose. Average \pm S.D. of 6 subjects.

Time (h)	Fraction	Cumulated
0-4	0.85 ± 0.58	0.85 ± 0.58
4-8	0.27 ± 0.18	1.12 ± 0.60
8-12	0.05 ± 0.04	1.19 ± 0.70
12-24	0.02 ± 0.03	1.19 ± 0.58

4. Discussion

4.1. Physical properties of glucosamine

Glucosamine is a small molecule (m.w. = 179.17), very soluble in water and soluble in hydrophilic organic solvents such as methanol. At 37 °C glucosamine has a pK_a of 6.91. This means that at pH 7.4, e.g. in the blood, 25% of glucosamine is ionized, and 75% is not ionized. At pH 6.8, e.g. in the small intestine, 46% is ionized and 54% is not ionized. At pH 1-3, e.g. in the stomach, glucosamine is completely ionized. The pK_a of glucosamine is therefore very favourable for an absorption from the small intestine and, in general for the crossing of biological barriers in the body. Only in the stomach glucosamine can exist in a very polar form, which is in most instances an obstacle for crossing cellular membranes.

All these properties candidate glucosamine as an easily absorbable and easily diffusible substance, as in fact it was experimentally found.

4.2. Binding of glucosamine to plasma proteins

It is important to know whether a substance binds with plasma albumins in order to understand the mechanisms of elimination and to speculate about possible interactions with other drugs. It was seen that in vitro glucosamine does not bind with plasma proteins of rat, dog or man. Interactions with other drugs on a pharmacokinetic basis seem therefore unlikely.

4.3. Pharmacokinetics of glucosamine

Immediately after i.v. administration to dogs of [^{14}C] glucosamine the radioactivity found in plasma is represented by glucosamine. This radioactivity is not bound to plasma proteins, is freely diffusible into the red cells (the plasma/red cell ratio of radioactivity is similar to the ratio of the concentrations of water in the two systems) and disappears from plasma according to a two-compartment open pharma-

cokinetic model. During this phase there is also the greatest urinary excretion of radioactivity, which can be identified as glucosamine. Between 30 and 60 min after administration, the radioactivity in plasma reaches a minimum. Then the radioactivity increases and reaches the peak around the 8th h. This radioactivity is no more due to glucosamine. It precipitates with the plasma proteins, it is not freely diffusible across the red cell membrane and does not give the specific reactions of glucosamine. Electrophoretically, it migrates with the α - and β -globulins of plasma, and is probably in the glycoproteins fractions. The liver plays an important role in the incorporation of the radioactivity in the plasma proteins. This could be proven experimentally in rats with a severe liver damage due to CCl_4 . In these animals the incorporation of radioactivity into the plasma proteins occurred with a slower rate than in normal rats.

The disappearance rate of the protein-incorporated radioactivity is very slow ($t_{1/2} = 2.9$ days) and is consistent with the turnover rate of plasma glycoproteins. During the first phase, i.e. during the phase with free glucosamine in plasma, the radioactivity diffuses rapidly into most organs. Biological barriers do not seem to exist, since radioactivity can be found e.g. in the brain in similar concentration as in plasma. Conversely, several organs and tissues show a capacity to concentrate glucosamine from plasma. The most important is the liver, which probably uses glucosamine for several biosynthetic processes. Also the kidney concentrates glucosamine and excretes it in the urine. Other tissues are also capable to concentrate and to retain glucosamine. Among the skeletal tissues, the articular cartilage has this capacity, as shown in Table 5 and by autoradiographic techniques in a previous study in the rat [11]. The incorporation into the articular cartilages is rapidly seen also after oral administration and represents the pharmacokinetic background for the therapeutic activity of glucosamine in some metabolic disorders of the cartilage.

After oral administration glucosamine is rapidly and well absorbed from the gastrointestinal tract, as seen also by Tesoriere et al. [12]. In fact, based on the fecal excretions of radioactivity after oral administration, the gastrointestinal absorption in the dog is 87% of the administered dose. The subsequent pharmacokinetic, distribution and metabolic pattern are fully compatible with those of free and protein-incorporated glucosamine found after i.v. administration.

4.4. Pharmacokinetic of glucosamine in man

The studies made with radioactive glucosamine in dogs and in rats show that the radioactivity remains for a very long time in several organs. It is therefore unethical to use radioactive glucosamine or other radioactive aminosugar derivatives in man. Pharmacokinetic studies in man are therefore possible only with cold glucosamine, even though the assay method has several limitations.

The results obtained administering to human volunteers cold glucosamine sulfate, i.v. or orally, and assaying glucosamine in plasma and urines with ion-exchange chromatography, show that the pharmacokinetics, distribution and metabolism of glucosamine in man does not substantially differ from that found in dogs and in rats.

5. Conclusions

After i.v. administration to the dog, glucosamine is found in plasma in a "free" form. In this form it is not protein-bound, is freely diffusible through biological membranes, and is selectively concentrated in some organs, first of all in the liver and in the kidney, but also in other tissues, e.g. in the articular cartilage. The free glucosamine disappears rapidly from plasma and is substituted by radioactivity incorporated into the plasma glycoproteins. This radioactivity follows the pharmacokinetic and metabolic pattern of plasma globulins. Glucosamine is mainly excreted in the urine. Only a small fraction of the administered glucosamine or of its derivatives are excreted in the feces. Notable fractions are metabolized to CO_2 and excreted in the expired air.

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After oral administration to the dog, glucosamine is rapidly and largely absorbed from the gastrointestinal tract. Its pharmacokinetics, distribution, metabolism and excretion pattern are consistent with those found after i.v. administration. The pharmacokinetic studies in man are limited by the fact that radioactive glucosamine cannot be administered for ethical reasons, and that the methods for assaying cold glucosamine have a low sensitivity and do not measure the products deriving from the metabolism of glucosamine. Even with these limitations, however, the results show that the pharmacokinetics of glucosamine in man after i.v. or oral administration does not differ substantially from that found with radioactive glucosamine in dogs and rats.

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Vergleich der Bioverfügbarkeit von zwei Diazepam-Zubereitungen

Klinische Vergleichsstudie zwischen einer neuen Handelszubereitung und einem Standardpräparat nach oraler und intramuskulärer Applikation

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Zusammenfassung: Die vorliegende Studie zur Pharmakokinetik von Diazepam untersuchte nach einem Crossover-Design an insgesamt 40 gesunden Probanden die Bioverfügbarkeit von Gewacalm® als neuem Testpräparat und einer weiteren Diazepam-Handelsform (DH) als Standardpräparat nach oraler und intramuskulärer Applikation. Der Vergleich der beiden Präparate erfolgte durch Bestimmung des Diazepam-Plasmaspiegels nach Gabe von jeweils 10 mg eines Präparates.

Im Vergleich der oralen Bioverfügbarkeit (N = 30) konnte im Hinblick auf Ausmaß und Geschwindigkeit der enteralen Resorption kein statistisch belegbarer Unterschied zwischen beiden Handelsformen gefunden werden. Im Vergleich der intramuskulären Bioverfügbarkeit (N = 10) zeigte sich ein Trend zur schnelleren und vollständigeren Resorption von Gewacalm gegenüber DH; dieser Trend war zwar statistisch

signifikant ($p < 0.05$), doch sollte wegen der relativ niedrigen Zahl der Probanden für den intramuskulären Vergleich dieses Ergebnis durch eine Replikationsstudie erhärtet werden.

Summary: Comparison of the Bioavailability of Two Diazepam Preparations/Clinical comparative study between a new commercial formulation and a standard preparation after oral and intramuscular application

The following pharmacokinetic study was aimed at a comparison of the bioavailability of two different preparations of diazepam (Gewacalm® and a further wellknown formulation). 40 healthy volunteers were subjected to a cross-over design in which plasma levels of diazepam were assayed after oral and intramuscular application of 10 mg of a preparation.

Single Dose Pharmacokinetics and Bioavailability of Glucosamine in the Rat

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Abstract Purpose: To study the pharmacokinetics of glucosamine following various routes of administration of the hydrochloride salt to rats and to locate the site of its first-pass metabolism. **Methods:** Rats were cannulated in the jugular vein and single intravenous, oral and intraperitoneal doses of 350 mg kg^{-1} were administered. Serial blood samples were collected and plasma glucosamine concentrations were determined using HPLC. **Results:** After intravenous administration, the apparent terminal half-life ($1.09 \pm 0.98 \text{ h}$), apparent steady state volume of distribution ($2.1 \pm 1.1 \text{ L kg}^{-1}$) and total body clearance ($2.61 \pm 0.81 \text{ L kg}^{-1} \text{ h}^{-1}$) were calculated. The peak plasma concentration, after oral administration, occurred approximately 30 min post-dose and the absolute bioavailability was 0.19. Glucosamine was completely bioavailable after intraperitoneal administration. **Conclusion:** Orally administered glucosamine is rapidly absorbed, highly distributed and efficiently cleared. The gut rather than liver is mainly responsible for the first pass metabolism since reduced bioavailability is observed after oral but not intraperitoneal doses.

INTRODUCTION

Osteoarthritis affects approximately 12% of the general population and the incidence increases with age (1). Current drug therapies, including acetaminophen and nonsteroidal anti-inflammatory drugs, do not slow or reverse the degenerative process in osteoarthritis. Glucosamine (2-amino-2-deoxy-D-glucose) has recently received a great deal of public attention as a treatment for osteoarthritis, prompting scientists to investigate its clinical usefulness and potential adverse effects (1). It has been proposed that glucosamine stops and possibly reverses the degenerative process in osteoarthritis (2). Pharmacokinetic studies of glucosamine in the rat, dog and man have been reported (3-5). However, these investigations have been conducted with radiolabeled drug and do not differentiate between

glucosamine and its metabolites and/or degradation products. We have applied a recently reported specific and sensitive HPLC-UV assay (6) to delineate the glucosamine pharmacokinetics. To explore the site of first pass-metabolism of glucosamine we followed the plasma time course of the drug after administration of single doses to rats via the intravenous (*i.v.*), intraperitoneal (*i.p.*) and oral (*p.o.*) routes.

MATERIALS AND METHODS

Materials

D-(+)-glucosamine hydrochloride, D-(+)-galactosamine hydrochloride and 1-naphthyl isothiocyanate (>95%) were purchased from Sigma Chemical Company (St. Louis, MO, USA). Methanol and acetonitrile were purchased from Caledon Laboratory Ltd (Georgetown, ON, Canada) while triethylamine and acetic acid were purchased from BDH Inc. (Toronto, ON, Canada). All chemicals and solvents were ACS analytical or HPLC grade. The styrene divinylbenzene quaternary ammonium solid-phase cartridges (200mg/4.0 mL; particle size range 45 - 150 μ) were obtained from Alltech Associates, Inc (Deerfield, IL, USA).

Animals

The study protocol was approved by the Health Sciences Animal Policy and Welfare Committee of the University of Alberta.

A group of five male Sprague-Dawley rats ($260 \pm 4 \text{ g}$) was used in *i.v.* and oral crossover studies with a two-day wash-out period between the two doses. The same cannula was used for bolus injection and for withdrawal of blood samples. Following bolus injection, the cannula was washed with normal saline solution to avoid the possibility of carry-over. A different group of six rats, in the same weight range, was used in the *i.p.* study. Rats were housed in cages and maintained in a controlled environment with free access to food and water.

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Procedure

Rats were anesthetized with single *i.p.* injections of sodium pentobarbital ($60 \text{ mg}\cdot\text{kg}^{-1}$), cannulated via the right jugular vein one day prior to drug administration and fasted overnight. Rats were administered exact volumes of glucosamine HCl solution ($200 \text{ mg}\cdot\text{mL}^{-1}$ in normal saline) to give single *i.v.* or *p.o.* doses of $350 \text{ mg}/\text{kg}$ as injections or gavages respectively. Blood samples (0.3 mL) were drawn at 0, 5, 10, 15, 30, 80, 90, 120, 240, 360, and 480 min post-dose and collected in heparinized tubes. They were centrifuged at 2000 g for 5 min and plasma was harvested and kept at -20° until analyzed.

Analytical Method

The determination of glucosamine in 0.1 mL plasma samples was performed using a previously described HPLC-UV method (6). On the chromatogram, galactosamine (internal standard) and glucosamine appeared at 26 and 29 min respectively. The assay was validated with a detection limit of $0.63 \text{ }\mu\text{g}\cdot\text{mL}^{-1}$ and a limit of quantification of $1.25 \text{ }\mu\text{g}\cdot\text{mL}^{-1}$. The response was linear over a concentration range of $1.25 - 400 \text{ }\mu\text{g}\cdot\text{mL}^{-1}$ and had an intra- and interday precision of $<11\%$. Glucosamine was found to be stable in rat plasma for at least one month at -20° . It was also stable for at least 24 h at room temperature during processing through the autosampler.

Pharmacokinetic Analysis

Pharmacokinetic indices of glucosamine were determined using non-compartmental analysis (WinNonlin version 3.1, Pharsight Corporation, CA, USA). Doses were normalized based on rat body weight. The terminal elimination rate constant, β , for the glucosamine concentration-time curve after *i.v.* administration was determined by the linear regression of at least three data points from the terminal portion of the plasma concentration-time plots. The area under the plasma concentration-time curve (AUC) was calculated using the trapezoidal rule up to the last measured plasma concentration, C_{last} . To the latter AUC was added C_{last}/β to calculate $\text{AUC}_{0-\infty}$. The total body clearance, Cl_{TB} , was determined by *i.v.* dose divided by $\text{AUC}_{0-\infty}$. The steady state volume of distribution, V_{dss} , was calculated from $V_{\text{dss}} = \text{dose}\cdot\text{MRT}/\text{AUC}_{0-\infty}$, where MRT is the mean residence time and AUMC is the area under the moment-time (7). The parameters were determined for each individual animal and the sample population averages were calculated. The observed peak plasma concentration (C_{max}) and the time-to-peak concentration (T_{max}) were

recorded. Absolute bioavailability (F) after oral doses was calculated as mean of the ratio $\text{AUC}_{\text{p.o.}}/\text{AUC}_{\text{i.v.}}$ for every individual rat, and for *i.p.* doses was based on the mean of $\text{AUC}_{\text{i.p.}}$ divided by mean of $\text{AUC}_{\text{i.v.}}$ for different groups.

Statistics

All the values are expressed as mean \pm standard deviation. Statistical significance between the means of groups was examined using ANOVA followed by the Duncan Multiple Range test ($p < 0.05$).

RESULTS

The plasma concentration-time profiles of glucosamine after *i.v.*, *i.p.*, and *p.o.* doses are shown in Figs.1 and 2. The mean pharmacokinetic indices are presented in Table 1. After *i.v.* administration, glucosamine declined in a multi-exponential fashion with a rapid initial distribution phase followed by a slower elimination phase (Fig. 1). Absorption after *i.p.* and *p.o.* administrations was rapid as indicated by the occurrence of mean peak plasma concentrations in less than 13 min with both routes of administrations (Table 1). There was no significant difference in the mean AUC values following *i.v.* and *i.p.* doses. Following *p.o.* doses, however, glucosamine demonstrated a significantly lower mean AUC as compared to *i.v.* and *i.p.* routes. The absolute bioavailability for *p.o.* doses was only 0.19 ± 0.21 .

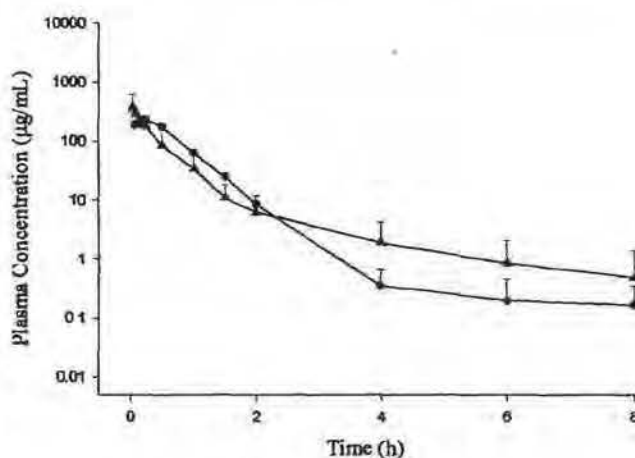


Figure 1: Mean glucosamine plasma concentration versus time curves after single *i.v.* bolus (▲) and *i.p.* (●) administration of $350 \text{ mg}\cdot\text{kg}^{-1}$ glucosamine HCl to rats. Concentrations below $1.23 \text{ }\mu\text{g}/\text{mL}$ were not used in the calculation of pharmacokinetic parameters. Error bars represent standard deviation of the mean ($n=5$ for *i.v.* and 6 for *i.p.*).

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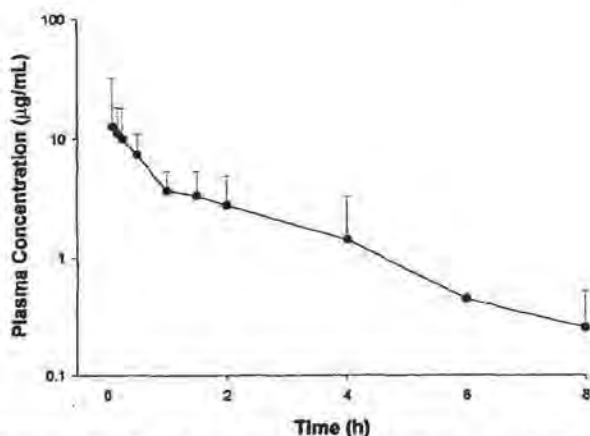


Figure 2: Mean glucosamine plasma concentration versus time curve after a single *p.o.* administration of 350 mg/kg⁻¹ glucosamine HCl to rats. Concentrations below 1.23 µg/mL were not used in the calculation of pharmacokinetic parameters. Error bars represent standard deviation of the mean (n=5).

Table 1: Pharmacokinetic indices of glucosamine after administration of 350 mg/kg⁻¹ glucosamine HCl to the rat.

Pharmacokinetic indices	Route of administration		
	<i>i.v.</i>	<i>i.p.</i>	<i>p.o.</i>
<i>T</i> _{max} (min)	nd	12.5 (2.7)	9.0 (4.18)
<i>C</i> _{max} (µg/mL ⁻¹)	nd	228 (41.8)	18.8 (15.9)*
<i>AUC</i> ₀₋₈ (µg.h/mL ⁻¹)	147 (53.7)	184 (18.7)	15.0 (8.0)**
<i>AUC</i> _{0-∞} (µg.h/mL ⁻¹)	150 (54.3)	187 (17.4)	22.9 (21.7)**
<i>t</i> _{1/2} (h)	1.09 (0.98)	0.69 (0.50)	2.16 (1.65)
<i>V</i> (L/kg ⁻¹)	5.7 (2.8)	nd	nd
<i>V</i> _d (L/kg ⁻¹)	2.12 (1.08)	nd	nd
<i>CL</i> (L/h ⁻¹ kg ⁻¹)	2.61 (0.81)	nd	nd
<i>F</i>		1.23	0.19 (0.21)

The values are the mean (SD), *n* = 5 for *i.v.* and *p.o.* and 6 for *i.p.*; nd, not determined; *significantly different from *i.p.*; **significantly different from *i.v.* and *p.o.*

DISCUSSION

Although glucosamine is considered an endogenous compound, we did not find detectable concentrations in blank plasma of the rat. Following *i.v.* administration, glucosamine is rapidly distributed and eliminated from the body. The multi-phasic appearance of the log-concentration-time curve indicates a multi-compartment model for the disposition of glucosamine. The observed mean steady state volume of distribution of 2.12 L/kg⁻¹ is rather large and indicates extensive distribution. Glucosamine is rapidly eliminated so that, for most samples, the plasma concentration falls below the detectable level of 0.63 µg/mL⁻¹ by 6 h post-dose. In addition to metabolic pathways, previous studies have suggested that glucosamine is taken up by bone and is incorporated into plasma proteins following administration to the rat and dog (3).

An earlier report (3) suggests a much longer terminal *t*_{1/2} for glucosamine in the rat. However, differentiation between the unchanged drug and its metabolites was not possible since radioabeled drug was administered in that study. Nevertheless, the true terminal *t*_{1/2} of glucosamine might have been longer than reported herein (Table 1) if we had been able to follow the plasma drug concentration for a longer period of time. Under our experimental conditions we were unable to measure drug concentrations beyond 6 h due to limitations in assay sensitivity. The objectives of our study, i.e., determination of glucosamine bioavailability and the possible site of first-pass metabolism, however, were achieved.

After oral administration, glucosamine is rapidly absorbed so that some samples taken five and ten minutes after administration contained the highest observed concentrations (Fig. 2). The *p.o.* doses, however, were only 21% bioavailable. This may be attributed to a low gastrointestinal absorption and/or extensive first-pass metabolism. For glucosamine, incomplete oral absorption may be ruled out since at least 82% of the administered radioactivity was found to be systemically available following oral administration of radiolabeled glucosamine to the rat (3). Hence, glucosamine is absorbed after oral administration as either unchanged glucosamine or breakdown products. Furthermore, hepatic first-pass metabolism may not be a major component of the overall clearance of glucosamine since *i.p.* doses exhibit complete bioavailability. Hepatic first-pass metabolism is expected to affect both *p.o.* and *i.p.* doses. The most likely explanation for the poor bioavailability of glucosamine, therefore, is a substantial loss in the gastrointestinal tract.

Our observations have been made following single doses of 350 mg/kg⁻¹ glucosamine hydrochloride. This dose may be considered rather high as compared with other drugs. However, the recommended dose of glucosamine for the treatment of osteoarthritis is 3 g daily (2). In addition, for pharmacokinetic studies, single oral doses of 6 g have been administered to human volunteers (4).

CONCLUSION

Glucosamine is rapidly absorbed, highly distributed and efficiently cleared. Since the low bioavailability of the drug is evident only after oral administration, the gut rather than liver is implicated for an apparent large first-pass effect.

ACKNOWLEDGMENTS

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Absorption, distribution and excretion of radioactivity after a single intravenous or oral administration of [^{14}C] glucosamine to the rat

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SUMMARY

Blood levels, tissue distribution and excretion patterns of radioactivity were studied in the rat after administration of [^{14}C] glucosamine sulphate by the intravenous or oral route. After intravenous administration, plasma radioactivity declined in the first 30 min, then increased, reaching a peak at the 2nd hour, and disappeared, with a half-disappearance time of 28 hours. The radioactivity diffused rapidly in the tissues. Higher levels than in plasma were reached in the liver and kidneys. There was early incorporation of radioactivity in the skeletal tissues (cartilage and bone). About 50% of the administered radioactivity was excreted with the expired CO_2 and about 35% with the urine. Faecal excretion was small (2% of the administered dose). After oral administration, radioactivity was quickly found in plasma, where it reached a peak at 4 hours. It then declined slowly, with biphasic kinetics. The tissue distribution, including uptake in the skeleton, repeated the pattern found after intravenous administration. There was only small faecal excretion, showing an almost complete bioavailability of glucosamine given orally, and the large excretion with the CO_2 (82%) showed that glucosamine is to a large extent broken down to smaller fragments. Autoradiographic studies confirmed the tissue distribution pattern and showed in more detail the tissue localization of radioactivity.

Key words: Glucosamine — pharmacokinetics — rat

INTRODUCTION

Glucosamine and the glucosamine-derived galactosamine are obligatory components of important macromolecules, such as the glycosaminoglycans of the connective, cartilaginous and mucosal tissues, of the membrane glycoproteins and glycolipids, of the plasma glycoproteins, etc. The normal source of glucosamine and of its acetylated derivative N-acetylglucosamine is the endogenous biosynthesis from glucose, but exogenous glucosamine, if available, becomes the preferential source for the biosynthesis of polyglycans, as shown already by the early studies of Rodén.¹¹ Rodén has also demonstrated that in the presence of glucosamine the polyglycan biosynthesis is enhanced by two mechanisms: the availability of the substrate for the macromolecules, and a stimulating effect on the incorporation of other essential substrates.

This double role of exogenous glucosamine prompted several investigations on the therapeutic usefulness of glucosamine, especially in diseases affecting the articulations, where disorders of polyglycan biosynthesis are involved.^{2,8} These studies demonstrated that glucosamine has a therapeutic effectiveness both in experimental lesions of the cartilage⁴ and in osteoarthritis.^{3,9,15,16}

Exogenous glucosamine, therefore, has an important biochemical and therapeutic role. Nevertheless, little seems to be known of its kinetics, distribution and excretion after parenteral or oral administration, therefore we started a series

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of investigations to study these aspects. Since glucosamine, upon introduction into the organism, is rapidly metabolized and/or incorporated in other substances, and since cold assay methods have a relatively low sensitivity, we made our investigations with [¹⁴C] uniformly labelled glucosamine, using the rat as the experimental animal.

MATERIALS AND METHODS

Animals

Animals used in this study were Sprague-Dawley outbred rats supplied by Charles River Laboratories, Calco, Italy. Before use, the animals were acclimatized for at least 1 week at a room temperature of 22±2°C and relative humidity of 60±10%. The animals were kept in individual Makrolon cages, fed with supplemented pellets (Altromin R) and had fresh tap water *ad libitum*.

Test substance

Uniformly labelled [¹⁴C] glucosamine was obtained from Amersham International Limited, with a specific activity of 1.23 mCi/mg, as hydrochloride, in a 0.615% solution in water. Before administration, glucosamine was properly diluted with unlabelled glucosamine sodium sulphate and NaCl to obtain a specific activity of 2 µCi/mg glucosamine. The solution to be administered had the stoichiometric composition of glucosamine sulphate. The doses expressed in mg/kg body weight refer to glucosamine sulphate.

Procedures for the counting technique experiments

An Intertechnique SL 32 counter was used. The dpm values were calculated using an external channel ratio method, with reference to a previously prepared standard quenching curve. Instagel, Dimilume 30, Carbosorb, Soluene 350 were all obtained from Packard Instruments (Milano).

The rats were fasted for 16 hours before treatment. Oral administration was made by gavage; intravenous administration was made via the caudal vein. A constant dose of 20 µCi/kg of [¹⁴C] glucosamine sulphate, carried in 10 mg/kg and in a volume of 2 ml/kg, was given. At the scheduled killing time the animals were anaesthetized with ether and exsanguinated from the abdominal aorta. The blood was heparinized and plasma and red cells separated by centrifugation. From each animal the following organs or tissues were removed: liver, kidneys, adrenals, brain, eyes, heart, ovaries and uterus (or testes), spleen, stomach (glandular part), intestine and intestinal contents, skeletal muscle, femoral head, cartilage, sternum, bone marrow, skin, subcutaneous fat, perirenal fat, and carcass. The organs and tissues were deep frozen until processing.

For both administration routes, the animals scheduled for sacrifice at 144 hours, upon having received the oral or intravenous administration, were placed in an all-glass metabolic cage to collect urine, faeces, and expired CO₂. Urine and faeces were collected daily, CO₂ was collected during the intervals 0 to 6, and 6 to 24 hours during the first day after administration, then urine was collected daily.

The killing times were scheduled as follows. Oral route: 0.25, 0.5, 1, 2, 4, 8, 16, 24, 48, 96 and 144 hours after administration; intravenous route: 0.17, 0.5, 1, 2, 4,

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8, 16, 24, 48, 96 and 144 hours after administration. At each time, 2 males and 2 females were sacrificed.

The total radioactivity in plasma and urine samples was counted directly in Instagel. Red blood cells were digested in Soluene 350 at 40°C in a shaking Dubnoff bath, bleached with a mixture of hydrogen peroxide (30%) and isopropanol and finally counted in Dimilume 30. The faeces excreted in the time periods considered were dried at 70°C under vacuum for 1 night and homogenized. Weighed aliquots were rehydrated, digested, bleached and counted as described for the red cell samples.

Tissues and organs were finely minced with scissors. Weighed aliquots of each tissue were digested, bleached and counted as described above. CO_2 in expired air was collected on Carbosorb. Measured aliquots of Carbosorb were counted in Dimilume 30 for the measure of [^{14}C] CO_2 . The carcasses were placed in sealed containers and digested in NaOH 10 N at 37°C for 5 days. Aliquots were neutralized with concentrated HCl, bleached and counted as described above.

Procedure for the whole body autoradiographies

Male rats were treated intravenously or orally with 20 $\mu\text{Ci/kg}$ [^{14}C] glucosamine sulphate carried in 10 mg/kg of the substance. The animals were sacrificed 10 minutes and 1, 4, 16, 48 and 72 hours after intravenous injection or 0.5, 1, 4, 16 and 48 hours after oral administration.

At the scheduled killing time the rat was anaesthetized with ether, laid on the back, held in position with a clamping device and immersed in a mixture of hexane and solid CO_2 at -70°C for 15 min. Once deep-frozen, the rat was embedded in a 2% water solution of carboxymethylcellulose and sagittally sectioned with an LKB 2220 cryomicrotome in 50 μm thickness slices. The sections were dehydrated on a transparent adhesive tape in cryostat at -20°C. The dehydrated sections were then exposed by contact to photographic films (Ultrofilm LKB, No-Screen Film Kodak) at -20°C. Once exposure had been completed (from 3 weeks to 2 months, according to the specimens) the film was developed by normal photographic procedures.

RESULTS

Pharmacokinetics of radioactivity after intravenous administration

Radioactivity levels in blood. The radioactivity levels in blood found after intravenous administration of 20 $\mu\text{Ci/kg}$ (10 mg/kg) [^{14}C] glucosamine sulphate were determined in plasma and in red cells at different times, individually on 2 males and 2 females each time. Since no substantial difference was found between males and females, the mean radioactivity found in the 4 animals was calculated and plotted in Figure I. The ratio between plasma and red cell radioactivity was also calculated.

The most important features of the radioactivity levels found were as follows: (i) there was an initial rapid decrease in plasma levels of radioactivity, with a minimum 30 min was after administration; (ii) there was a subsequent increase in plasma radioactivity, up to a maximum at the 2nd hour after administration; (iii) after the 2nd hour, the plasma radioactivity disappeared slowly. From 8 hours

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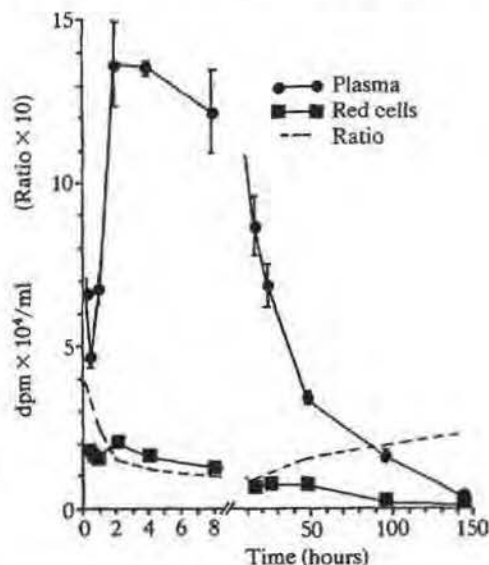
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Figure I. Radioactivity in plasma and in red cells after 20 μ Ci/kg (4.44×10^7 dpm/kg) of [¹⁴C] glucosamine sulphate given intravenously: mean (\pm S.E.M.) values from 4 animals

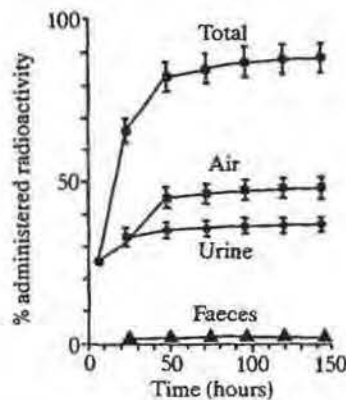


onwards, the disappearance rate was close to a first-order linear kinetics, with a half-disappearance time of 28 hours ($r=0.99$); (iv) the ratio between plasma and red cells radioactivity changed notably during the observation time.

These results suggest that the radioactivity is not due to glucosamine, but derives from chemical entities which change in time, i.e. that glucosamine is metabolized and probably incorporated into plasma proteins.

Radioactivity in urine, faeces and expired CO₂. At each time, the data of 4 animals, 2 males and 2 females, were available. Since there was no significant difference between males and females, the mean of the 4 results was plotted in Figure II.

Figure II. Cumulative excretion of radioactivity with expired air, urine and faeces after intravenous administration of [¹⁴C] glucosamine sulphate: mean (\pm S.E.M.) percentages of administered radioactivity from 4 animals



About half of the radioactivity was excreted with the CO₂ of the expired air, showing that a substantial amount of the administered glucosamine was completely broken down and utilized for energetic processes. Almost 40% of radioactivity was excreted with the urine. Of this amount, 90% was excreted during the first 24 hours. Only a small amount, i.e. 2% of the administered dose, was excreted with the faeces. Total excretion with CO₂, urine and faeces, after 144 hours, was found to be 88% of the administered radioactivity. At this time in the animals a further 2.2% of administered radioactivity was found in the organs and another 5.5% in the carcasses, so that 144 hours after administration the total recovery of radioactivity amounted to 96%.

Radioactivity in organs and tissues. At each time, data from 2 males and 2 females were available. Since there was no substantial difference between sexes, the mean values for the 4 sets of data were calculated (Table 1).

The most important features of the distribution of radioactivity in tissues were as follows: (i) radioactivity of [¹⁴C] glucosamine sulphate entered quickly into the tissues. In fact, 10 min after administration radioactivity was found already in all organs and tissues, including the cartilages; (ii) in the first 2 hours radioactivity was concentrated in the liver and kidney, i.e. in the organs involved in metabolism and excretion. In these organs during these periods radioactivity was 4 to 5-times higher than in plasma. After the 2nd hour, the radioactivity in the liver and kidney behaved parallel to that in plasma; (iii) in all other organs and tissues the radioactivity was lower than that of plasma; (iv) the maximum concentration of radioactivity in the different organs was normally reached within the first 8 hours. After 24 hours, the radioactivity decreased, with a half-disappearance time of 20 to 40 hours. A similar behaviour is found in plasma.

Pharmacokinetics of radioactivity after oral administration

Radioactivity levels in blood. The radioactivity levels in blood found after an oral dose (by gavage) of 20 µCi/kg (10 mg/kg) of [¹⁴C] glucosamine sulphate was assayed in plasma and in red cells of the animals sacrificed at different times, individually on 2 males and 2 females at each time. Since no substantial differences were found between males and females, the mean of the radioactivities found in the 4 animals was calculated and plotted in Figure III.

The following features are noteworthy: (i) fifteen minutes after administration, the radioactivity was already present in plasma and in red cells; it then increased rapidly, reaching the peak at the 4th hour. After the 8th hour, the radioactivity decreased, with a half-disappearance time of 17.7 hours between the 8th and the 48th hour, and of 46 hours after the 48th hour. The correlation between calculated and found radioactivity levels was very good ($r=0.92$); (ii) during the first 8 hours the radioactivity in the red cells was parallel to that in plasma, with a red cell/plasma ratio of 0.3 to 0.4. After the 8th hour, the radioactivity in the red cells behaved independently, with a relative increase compared to the plasma radioactivity. After the 24th hour, the radioactivity in the red cells increased also in absolute terms, showing that plasma and red cell radioactivity originate from different substances.

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Table 1. Radioactivity of tissues and organs after intravenous administration of [¹⁴C] glucosamine sulphate: mean (±S.E.M.) dpm × 10⁴/g wet tissue

Organ or tissue	Time after administration (hours)											
	0.17	0.5	1	2	4	8	16	24	48	96	144	
Liver	22.23 (0.72)	24.94 (2.05)	25.31 (2.17)	19.88 (1.73)	11.02 (0.43)	8.07 (0.73)	6.30 (0.67)	4.85 (0.34)	2.35 (0.04)	1.46 (0.07)	0.71 (0.04)	
Kidney	28.79 (6.54)	11.18 (0.56)	9.98 (0.84)	9.45 (0.55)	6.11 (1.08)	7.07 (0.66)	6.56 (0.19)	5.95 (0.32)	3.82 (0.22)	2.50 (0.29)	1.55 (0.05)	
Lung	3.78 (0.21)	2.09 (0.10)	1.98 (0.13)	2.92 (0.32)	3.34 (0.20)	3.65 (0.29)	2.59 (0.31)	2.16 (0.15)	1.54 (0.03)	1.21 (0.08)	0.80 (0.08)	
Stomach wall (glandular part)	2.02 (0.36)	1.61 (0.14)	0.99 (0.11)	1.19 (0.03)	1.34 (0.08)	1.24 (0.24)	1.35 (0.07)	1.29 (0.10)	0.90 (0.07)	0.74 (0.07)	0.29 (0.05)	
Stomach wall (rumen)	1.91 (0.29)	1.85 (0.21)	1.59 (0.14)	2.08 (0.19)	2.18 (0.20)	2.60 (0.33)	1.91 (0.09)	1.56 (0.09)	0.94 (0.15)	0.65 (0.08)	0.30 (0.02)	
Intestine wall	3.55 (0.72)	3.32 (0.38)	4.42 (0.56)	3.90 (0.74)	3.83 (0.35)	3.04 (0.42)	1.96 (0.18)	2.07 (0.07)	1.09 (0.19)	0.86 (0.04)	0.31 (0.03)	
Intestinal contents	1.53 (0.40)	1.14 (0.49)	2.01 (0.63)	3.62 (0.91)	5.10 (0.74)	4.00 (0.77)	2.38 (0.38)	1.64 (0.16)	0.73 (0.26)	0.26 (0.06)	0.09 (0.02)	
Brain	4.73 (0.71)	4.88 (0.36)	6.02 (0.49)	5.74 (0.19)	4.64 (0.20)	3.74 (0.19)	3.35 (0.12)	3.27 (0.25)	2.07 (0.13)	2.04 (0.14)	1.74 (0.15)	
Eye	2.96 (0.49)	2.23 (0.13)	2.40 (0.24)	1.96 (0.09)	1.44 (0.04)	1.78 (0.12)	1.37 (0.07)	1.70 (0.08)	1.01 (0.14)	0.92 (0.11)	0.77 (0.04)	
Adrenals	1.93 (0.21)	1.59 (0.25)	2.09 (0.14)	3.15 (0.14)	3.97 (0.57)	5.24 (0.61)	4.82 (0.35)	6.59 (0.75)	3.07 (0.37)	2.93 (0.40)	1.37 (0.12)	
Testes*	1.36	0.79	0.95	1.28	2.38	2.13	2.01	1.49	0.73	0.52	0.27	
Ovaries*	2.81	1.83	2.52	3.91	4.11	4.60	4.57	4.13	2.62	1.64	0.98	
Uterus*	4.94	2.13	2.24	1.89	1.56	1.84	2.24	2.99	1.94	1.63	0.93	
Heart	2.05 (0.49)	1.31 (0.14)	1.48 (0.30)	2.21 (0.24)	2.44 (0.10)	2.15 (0.27)	1.78 (0.05)	1.47 (0.16)	1.30 (0.11)	1.62 (0.40)	0.52 (0.05)	
Skeletal muscle	0.93 (0.14)	0.75 (0.12)	0.49 (0.05)	0.53 (0.05)	0.61 (0.10)	0.87 (0.06)	0.56 (0.09)	0.53 (0.10)	0.50 (0.03)	0.31 (0.07)	0.22 (0.04)	
Spleen	2.60 (0.45)	1.94 (0.19)	2.67 (0.55)	2.49 (0.12)	2.79 (0.28)	2.82 (0.15)	3.05 (0.33)	2.81 (0.15)	1.62 (0.17)	1.41 (0.05)	0.59 (0.06)	
Femoral cartilage	3.38 (0.67)	1.87 (0.09)	1.41 (0.14)	1.99 (0.12)	2.14 (0.12)	2.40 (0.28)	2.13 (0.21)	2.00 (0.20)	1.04 (0.08)	0.96 (0.06)	0.56 (0.11)	
Sternum	2.68 (0.47)	1.33 (0.11)	1.21 (0.07)	1.58 (0.10)	1.43 (0.12)	1.47 (0.19)	1.40 (0.09)	1.32 (0.04)	1.17 (0.11)	1.15 (0.12)	0.50 (0.07)	
Bone marrow	1.94 (0.39)	1.50 (0.06)	1.77 (0.07)	2.99 (0.16)	3.50 (0.47)	3.81 (0.32)	3.22 (0.28)	2.87 (0.13)	1.45 (0.12)	1.25 (0.16)	0.53 (0.04)	
Subcutaneous fat	1.96 (0.28)	1.30 (0.12)	0.70 (0.14)	0.56 (0.09)	0.63 (0.11)	1.07 (0.19)	1.11 (0.06)	1.17 (0.14)	0.62 (0.03)	0.58 (0.08)	0.52 (0.08)	
Perirenal fat	2.89 (0.90)	1.99 (0.30)	1.65 (0.38)	0.98 (0.23)	1.22 (0.33)	0.87 (0.15)	2.25 (0.46)	1.84 (0.55)	0.82 (0.06)	0.80 (0.05)	0.78 (0.08)	
Skin	3.16 (0.53)	1.73 (0.02)	1.09 (0.06)	1.19 (0.11)	1.07 (0.10)	1.42 (0.16)	1.26 (0.16)	1.36 (0.17)	1.00 (0.09)	0.89 (0.05)	0.42 (0.04)	
Carcass											5.52 (0.23)	
Plasma (dpm × 10 ⁴ /ml)	6.61	4.60	6.67	13.67	13.57	12.28	8.70	6.92	3.42	1.67	0.35	

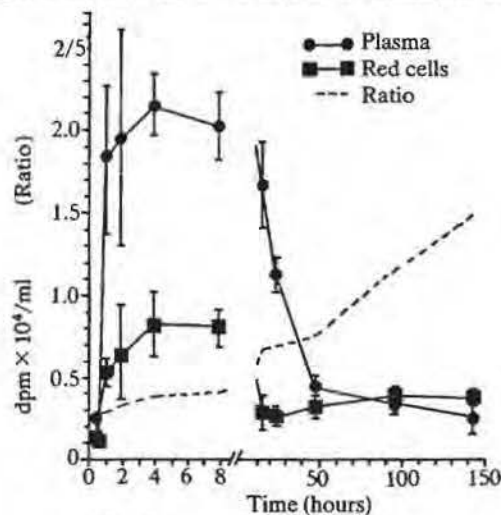
*Mean values from 2 animals

Radioactivity in urine, faeces and expired CO₂. At each time the data of 4 animals, 2 males and 2 females, were available. Since there was no significant difference

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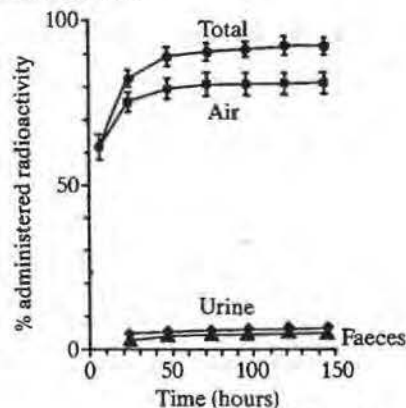
Figure III. Radioactivity in plasma and in red cells after 20 $\mu\text{Ci/kg}$ (4.44×10^7 dpm/kg) of [^{14}C] glucosamine sulphate after oral administration: mean (\pm S.E.M.) values from 4 animals



Note: the ordinates are expanded with respect to Figure I

between males and females, the mean of the 4 results was plotted in Figure IV. More than 61% of the administered radioactivity was recovered in CO_2 . In the first 6 hours, the radioactivity amounted to 61% of that administered. These data show the high degree of metabolism of [^{14}C] glucosamine sulphate after oral administration. About 6% of the administered dose was excreted into the urine and about 5% with the faeces. Taking into account the residual radioactivity in organs and in the carcass at the 144th hour, the recovery of radioactivity amounted to 98%.

Figure IV. Cumulative excretion of radioactivity with expired air, urine and faeces after oral administration of [^{14}C] glucosamine sulphate: mean (\pm S.E.M.) percentages of administered radioactivity from 4 animals



^{14}C (dpm/kg) of
from 4 animals

Radioactivity in organs and tissues. Data from 4 animals, 2 males and 2 females, were available at each sampling time. Since there was no significant difference between males and females, the mean of the 4 animals was calculated, as shown in Table 2.

Table 2. Radioactivity of tissues and organs after oral administration of [^{14}C] glucosamine sulphate: mean (\pm S.E.M.) dpm $\times 10^4$ /g wet tissue

Organ or tissue	Time after administration (hours)										
	0.25	0.5	1	2	4	8	16	24	48	96	144
Liver	0.82 (0.12)	1.46 (0.19)	5.63 (1.18)	5.72 (2.62)	4.47 (1.06)	5.02 (0.92)	3.41 (0.33)	2.50 (0.23)	4.43 (0.36)	0.58 (0.05)	0.57 (0.04)
Kidney	0.37 (0.08)	0.65 (0.09)	1.98 (0.41)	2.05 (0.52)	2.30 (0.25)	2.12 (0.19)	2.01 (0.14)	1.79 (0.07)	0.89 (0.14)	0.93 (0.10)	0.81 (0.04)
Lung	0.11 (0.03)	0.35 (0.04)	1.31 (0.30)	1.12 (0.48)	1.61 (0.20)	1.79 (0.18)	1.26 (0.06)	1.27 (0.07)	0.88 (0.11)	1.29 (0.34)	1.11 (0.35)
Stomach wall (glandular part)	100.43 (16.62)	38.30 (13.47)	29.99 (14.26)	3.92 (1.03)	1.63 (0.43)	1.55 (0.24)	1.19 (0.20)	1.09 (0.08)	0.56 (0.10)	0.41 (0.09)	0.41 (0.02)
Stomach wall (rumen)	148.10 (22.99)	65.70 (22.66)	33.42 (14.61)	20.82 (16.77)	3.07 (0.93)	2.69 (0.39)	2.01 (0.15)	1.60 (0.15)	0.69 (0.22)	0.50 (0.02)	0.46 (0.01)
Intestine wall	62.74 (7.63)	22.97 (3.44)	15.99 (1.64)	17.48 (6.75)	4.33 (0.84)	3.59 (0.39)	2.88 (0.21)	2.68 (0.20)	0.92 (0.08)	0.62 (0.09)	0.60 (0.12)
Intestinal contents	35.75 (6.97)	38.03 (9.33)	24.76 (10.69)	58.04 (9.05)	44.86 (13.78)	17.43 (6.94)	13.96 (2.99)	10.16 (2.18)	0.47 (0.07)	0.13 (0.03)	0.06 (0.01)
Brain	0.13 (0.03)	0.19 (0.02)	2.04 (0.54)	1.95 (1.02)	2.50 (0.64)	1.91 (0.19)	1.15 (0.16)	1.16 (0.14)	0.32 (0.02)	0.39 (0.10)	0.51 (0.12)
Eye	0.09 (0.03)	0.26 (0.08)	1.10 (0.42)	1.11 (0.58)	1.13 (0.33)	0.95 (0.23)	0.58 (0.10)	0.52 (0.04)	0.22 (0.01)	0.19 (0.03)	0.18 (0.06)
Adrenals	0.13 (0.04)	0.12 (0.03)	1.78 (0.56)	1.80 (0.83)	3.85 (0.79)	5.17 (0.38)	3.78 (0.53)	2.27 (0.31)	2.23 (0.30)	1.78 (0.24)	0.75 (0.01)
Testes*	0.03	0.06	0.61	0.97	1.26	1.15	0.91	0.84	0.26	0.22	0.20
Ovaries*	0.17	0.21	1.28	0.67	2.03	2.41	1.71	1.78	0.78	0.97	0.70
Uterus*	0.19	0.27	1.32	0.74	1.75	1.55	1.00	1.14	0.55	0.55	0.42
Heart	0.09 (0.04)	0.07 (0.03)	0.74 (0.20)	0.68 (0.29)	1.50 (0.23)	1.12 (0.10)	0.57 (0.05)	0.61 (0.03)	0.35 (0.03)	0.42 (0.07)	0.71 (0.21)
Skeletal muscle	0.05 (0.02)	0.08 (0.02)	0.47 (0.11)	0.44 (0.24)	0.55 (0.07)	0.61 (0.05)	0.41 (0.03)	0.35 (0.07)	0.26 (0.02)	0.28 (0.03)	0.20 (0.03)
Spleen	0.07 (0.01)	0.11 (0.01)	1.23 (0.38)	1.19 (0.56)	2.25 (0.32)	2.94 (0.17)	1.93 (0.15)	1.81 (0.27)	0.79 (0.08)	0.63 (0.06)	0.50 (0.11)
Femural cartilage	0.13 (0.03)	0.17 (0.02)	1.12 (0.30)	1.70 (0.80)	1.61 (0.30)	2.07 (0.11)	1.26 (0.15)	1.23 (0.14)	0.51 (0.06)	0.36 (0.04)	0.30 (0.06)
Sternum	0.25 (0.16)	0.10 (0.01)	1.00 (0.23)	0.93 (0.50)	1.53 (0.20)	1.66 (0.26)	1.10 (0.09)	1.10 (0.09)	0.74 (0.09)	0.34 (0.05)	0.37 (0.09)
Bone marrow	0.32 (0.12)	0.22 (0.04)	1.89 (0.24)	2.13 (0.67)	3.21 (0.59)	4.02 (1.21)	3.22 (0.56)	3.16 (0.54)	2.53 (1.20)	0.78 (0.11)	0.96 (0.19)
Subcutaneous fat	0.15 (0.04)	0.12 (0.02)	0.98 (0.24)	0.71 (0.43)	1.11 (0.21)	1.13 (0.19)	0.92 (0.07)	0.89 (0.09)	1.06 (0.28)	1.43 (0.43)	1.30 (0.54)
Perirenal fat	0.22 (0.05)	0.39 (0.11)	1.67 (0.44)	1.83 (0.87)	2.19 (0.51)	2.13 (0.14)	1.58 (0.06)	2.09 (0.33)	1.78 (0.20)	2.13 (0.55)	2.13 (0.88)
Skin	0.30 (0.17)	0.19 (0.07)	0.89 (0.35)	0.77 (0.50)	1.07 (0.27)	1.02 (0.09)	0.86 (0.18)	1.56 (0.81)	0.64 (0.05)	0.63 (0.12)	0.40 (0.11)
Plasma (dpm $\times 10^4$ /ml)	0.22	0.27	1.82	1.95	2.14	2.02	1.66	1.11	0.44	0.34	0.25

*Mean values from 2 animals

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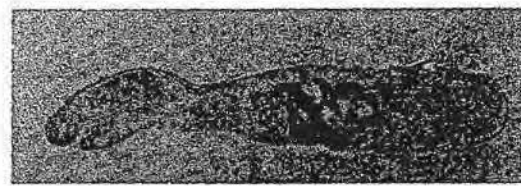
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Figure V. Autoradiography of a sagittal section of a rat after intravenous administration of [^{14}C] glucosamine sulphate (20 $\mu\text{Ci/kg}$ body weight), and an enlargement of the femoral head showing details of the distribution of radioactivity in bone and cartilages: 10 minutes after injection

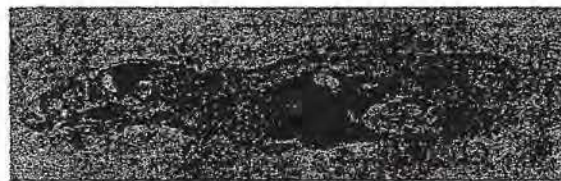


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1 cm

Figure VI. Autoradiography of a sagittal section of a rat after intravenous administration of [^{14}C] glucosamine sulphate (20 $\mu\text{Ci/kg}$ body weight), and an enlargement of the femoral head showing details of the distribution of radioactivity in bone and cartilages: 1 hour after injection



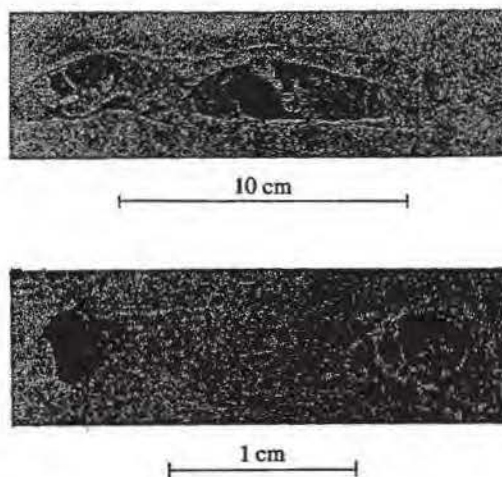
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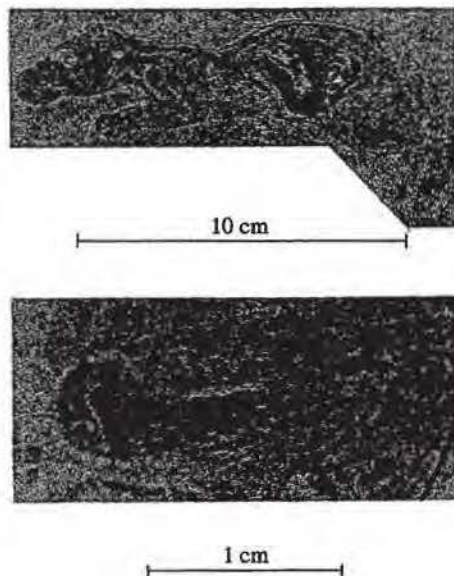
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Figure VII. Autoradiography of a sagittal section of a rat after intravenous administration of [^{14}C] glucosamine sulphate (20 $\mu\text{Ci/kg}$ body weight), and an enlargement of the femoral head showing details of the distribution of radioactivity in bone and cartilages: 48 hours after injection



s administration of
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Figure VIII. Autoradiography of a sagittal section of a rat 4 hours after oral administration of [^{14}C] glucosamine sulphate (20 $\mu\text{Ci/kg}$ body weight), and an enlargement of the humerus showing details of the distribution of radioactivity in bone and cartilages



Also after oral administration, the radioactivity tended to concentrate in the metabolizing and excreting organs, such as the liver and the kidney, and remained higher than in plasma during the whole time of the experiment. In the liver, the peak was reached at the 2nd hour after administration, in the kidneys at the 4th hour. Worth noting is the early presence of radioactivity in all organs, including the cartilage, where after the 8th hour the radioactivity was approximately equal to that found in plasma.

Autoradiographies after intravenous administration

Ten minutes after intravenous administration the radioactivity was already concentrated in the liver, in the kidneys, in the cartilage and epiphysis of the femoral head and in other extravascular tissues (Figure V). This distribution pattern was seen more clearly 1 hour after administration (Figure VI) and was still very evident 48 hours after administration (Figure VII).

Autoradiographies after oral administration

Thirty minutes after oral administration the radioactivity was already marked in the liver and the kidneys. The extra-gastro-intestinal radioactivity increased after 1 hour and even more after 4 hours (Figure VIII). At this time and after 16 and 48 hours, the distribution pattern of radioactivity was very similar to that seen after intravenous administration.

DISCUSSION

After intravenous administration of [^{14}C] uniformly labelled glucosamine, the plasma radioactivity decreased rapidly during the first 30 minutes. In this phase, the plasma radioactivity is probably due to free glucosamine, i.e. to the injected substance. During this phase, glucosamine was taken up in large amounts in the liver, and at the 30th minute after administration 20% of the administered radioactivity was captured by this organ, which is probably the main one responsible for the metabolism and biotransformation of exogenous glucosamine. These results are consistent with those of Lo *et al.*⁷ and of Akamatsu *et al.*,¹ who have studied the incorporation in the liver and the biotransformation of exogenous glucosamine. After 30 minutes, the radioactivity increased in plasma, reaching a peak at the 2nd hour; it then decreased very slowly, with a linear disappearance constant of 0.024 hours \exp^{-1} . In this phase, the radioactivity probably originates from the plasma proteins, in which the exogenous glucosamine or fragments from it are incorporated.¹ The radioactivity was also rapidly incorporated in other tissues and retained there for a long time. Notable among these were the cartilages and the growing bone, which rapidly take up circulating glucosamine and use it for the biosynthesis of the proteoglycans of the extracellular organic matrix.^{10,14} Taking into account that the skeleton (bones and cartilages) represent a substantial amount of the mass of the body, it can be calculated that about 30% of the administered glucosamine is taken up by the skeletal tissues for the metabolic, energetic and biotransformation processes. Also, other tissues with a great muco-

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concentrate in the
liver, and remained
there. In the liver, the
radioactivity in the
kidneys at the 4th
hour, including
radioactivity in
other organs, including
approximately equal

radioactivity was already con-
sistent with the femoral
distribution pattern was
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radioactivity or fragments from
radioactivity incorporated in other
radioactivity where the cartilages
radioactivity mine and use it for
radioactivity organic matrix.^{10,14}
radioactivity present a substan-
radioactivity about 30% of the
radioactivity for the metabolic,
radioactivity with a great muco-

polysaccharide content (mucosa, skin) are involved in the uptake of glucosamine, as shown by our results and those reported by other investigators.^{5,6,12}

Notable was the amount of radioactivity excreted in the expired air, amounting to half of the administered dose, a process which continued during the first 48 hours after administration. This represents probably the fraction of glucosamine utilized for energetic purposes and as -NH₂ donor substrate. The amount excreted in the urine was smaller (35% of the administered dose). Urinary excretion was almost completed in the first 24 hours. In other experiments (not reported here) it was seen that the urinary excretion of radioactivity was practically completed in the first 3 to 6 hours after administration. Faecal excretion of radioactivity was negligible and, under the present experimental conditions, largely an artefact due to contamination with urine.

After oral administration of [¹⁴C] glucosamine, the radioactivity was already present in plasma after 15 minutes. It reached its peak at the 4th hour and then declined progressively, with a disappearance constant of 0.039 hrs exp-1 between the 8th and 48th hour, and of 0.004 hrs exp-1 after the 48th hour. The plasma disappearance was very slow, and the same slow disappearance was seen in the tissues. Also notable was the dissociation between the plasma and the erythrocyte radioactivity, the latter being probably linked to the membrane polysaccharides.

A large amount of the administered radioactivity was found in the expired CO₂ during the first 24 to 48 hours after administration. This was parallel to the incorporation in the liver (4.73% of the administered dose at the 8th hour); however, it was lower than after intravenous administration. The faecal excretion of radioactivity was very low (5% of the administered dose), showing the good absorption of exogenous glucosamine from the gastro-intestinal tract, which is accomplished without any metabolic breakdown of the molecule.¹³ The tissue distribution pattern of radioactivity was similar to that found after intravenous administration, apart from the time course differences inherent to the two administration routes. This includes also the incorporation in bone and in cartilage, which was very evident in the autoradiographs taken 4 hours after administration and persisted for the following 44 hours.

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Pharmacokinetics of Glucosamine in Man

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Summary

The pharmacokinetics of glucosamine sulfate (CAS 29031-19-4) was investigated in 6 healthy male volunteers (2 per administration route) using ¹⁴C uniformly labelled glucosamine sulfate and administering it in single dose by intravenous (i.v.), intramuscular (i.m.) or oral route. The results show that after i.v. administration the radioactivity due to glucosamine appears in plasma and is rapidly eliminated, with an initial $t_{1/2}$ of 0.28 h. 1-2 h after administration the radioactivity due to glucosamine disappears almost completely and is replaced by a radioactivity originating from plasma proteins, in which glucosamine or its metabolites are incorporated. This radioactivity reaches a peak after 8-10 h and then declines with a $t_{1/2}$ of 70 h. About 28% of the administered radioactivity is recovered in the urine of the 120 h following the administration and less than 1% is recovered in the feces.

After i.m. administration similar pharmacokinetic patterns are observed.

After oral administration a proportion close to 90% of glucosamine sulfate is absorbed. Free glucosamine is not detectable in plasma. The radioactivity incorporated in the plasma proteins follows pharmacokinetic patterns which are similar to those after i.v. or i.m. administration, but its concentration in plasma is about 5 times smaller than that after parenteral administration. The AUC after oral administration is 26% of that after i.v. or i.m. administration. The smaller plasma levels of radioactivity after oral administration are probably due to a first pass effect in the liver which metabolizes a notable proportion of glucosamine into smaller molecules and ultimately to CO₂, water and urea.

The results confirm previous investigations in rats and dogs showing that also in man glucosamine sulfate is a prodrug for glucosamine that is well absorbed after oral administration and that, after i.v., i.m. or oral administration, diffuses into several tissues, including bones and articular cartilages.

Zusammenfassung

Pharmakokinetik von Glucosamin beim Menschen
An 6 gesunden männlichen Freiwilligen (jeweils 2 pro Art der Verabreichung) wurde die Pharmakokinetik von

Glucosaminsulfat (CAS 29031-19-4) untersucht, wobei ¹⁴C-markiertes Glucosaminsulfat intravenös (i.v.), intramuskulär (i.m.) oder oral als Einzeldosis angewendet wurde. Die Ergebnisse zeigen, daß die Radioaktivität im Plasma nach i.v. Anwendung von markiertem Glucosamin schnell mit einer initialen Halbwertszeit von 0,28 h eliminiert wird. 1-2 h nach der Anwendung verschwindet die Radioaktivität meist ganz und erscheint in den Plasmaproteinen, in die Glucosamin oder seine Metaboliten inkorporiert wurden. Nach 8-10 h erreicht die Radioaktivität den Spitzenwert, der dann mit einer Halbwertszeit von 70 h sinkt. Ungefähr 28% der verabreichten Radioaktivität werden im Sammelurin über 120 h nach der Anwendung gefunden; im Stuhl ist weniger als 1% zu finden.

Bei i.m. Anwendung wurde ein ähnliches pharmakokinetisches Profil beobachtet.

Bei oraler Anwendung werden bis zu 90% Glucosaminsulfat resorbiert, wobei kein freies Glucosamin im Plasma zu finden ist. Die in die Plasmaproteine inkorporierte Radioaktivität hat ein ähnliches pharmakokinetisches Profil wie die nach i.v. oder i.m. Anwendung, die Plasmakonzentration ist jedoch etwa 5mal geringer als nach parenteraler Anwendung. Die AUC beträgt bei oraler Anwendung 26% der AUC bei i.v. oder i.m. Anwendung. Die geringeren Plasmaspiegel der Radioaktivität bei oraler Anwendung sind wahrscheinlich auf einen First-pass-Effekt in der Leber zurückzuführen, die einen beträchtlichen Teil von Glucosamin in kleinere Moleküle und letztlich zu Kohlensäure, Wasser und Harnstoff metabolisiert.

Die Ergebnisse bestätigen frühere Untersuchungen an Ratten und Hunden und zeigen, daß Glucosaminsulfat auch beim Menschen ein Prodrug des Glucosamins ist, das bei oraler Anwendung gut resorbiert wird und sich bei i.v., i.m. und oraler Anwendung in die verschiedenen Gewebe, einschließlich Knochen und Gelenknorpel, verteilt.

Key words: Anti-inflammatories, non-steroidal
CAS 29031-19-4 - Glucosamine sulfate, pharmacokinetics

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1. Introduction

Glucosamine is an autotrophic aminomonosaccharide synthesized in our body from glucose and used for the biosynthesis of glucosaminoglycans and glycoproteins [2]. Glucosamine can be obtained also by chemical synthesis as pure substance and this exogenous glucosamine is used under the form of glucosamine sulfate (GS, CAS 29031-19-4) for the therapy of osteoarthritic disorders [3, 5]. GS can be administered by oral, intramuscular (i.m.) or intravenous (i.v.) route. After oral administration it is rapidly absorbed and splitted into D-glucosamine (DG) and sulfate ion. The same process occurs after i.m. or i.v. administration. DG is a small molecule with a m.w. of 179.17, is a well defined and simple chemical entity, is very soluble in water and has a pK_a of 6.91. Due to these physical properties DG is easily diffusible in the body compartments. DG has a special tropism for cartilaginous and bone tissues where it probably represents the preferred substrate for the biosynthesis of the proteoglycans of the cartilage matrix [7, 8].

The pharmacokinetics of DG is difficult to investigate, first because DG is an autotrophic substance rapidly utilized by the body for the biosynthesis of other normal constituents and is therefore not recoverable as parent compound, and second because the assay methods presently available for DG have an insufficient sensitivity to detect the small quantities of DG occurring in the biological media after administration of therapeutic doses of GS.

Actually the pharmacokinetics of GS could be studied in the rat and in the dog using ^{14}C uniformly labelled DG [7, 8], and this is to our knowledge still the only method practically available for pharmacokinetic investigations on DG. Obviously, in man the use of radiolabelled DG suffers from many ethical and technical limitations.

Nevertheless we were compelled to make human pharmacokinetic studies for regulatory reasons and for evaluating the consistency of the results obtained in animals with the pharmacokinetic features of DG in man. It was necessary, however, to restrict the study to an extremely small number of subjects (two for each administration route), and to administer a single dose only, with the minimum possible amount of ^{14}C -DG.

2. Subjects, materials and methods

2.1. Subjects

Enrolled were 6 male healthy volunteers who had given in writing their consent after having received a complete information on the goal of the study, the risks and the discomforts involved, and the assurance that they could exit the study at any time upon their request. Before enrolment the health status was checked by medical history, physical examination, routine hematology, blood chemistry and urinalysis. The demographic data of the volunteers are given in Table 1.

Table 1: Demographic data of the subjects.

Route	Subject no.	Age (years)	Weight (kg)	Height (cm)	Broca index
I.v.	1	31	94	179	119
	2	32	72	174	97
I.m.	3	38	85	171	120
	4	23	67	167	100
Oral	5	21	90	180	113
	6	23	78	182	95
Average		29.7	81.0	176	107
SD		5.8	10.5	6	11
Range		23-38	67-94	167-182	95-120

2.2. Investigational preparations and administration

Uniformly labelled ^{14}C -D-glucosamine was obtained from Amersham International Limited (Amersham U.K.), with a specific radioactivity of 1.23 mCi/mg. The product was supplied as hydrochloride in a 0.615 % aqueous solution. The solution was diluted with unlabelled GS and water to obtain the final preparations with the desired radioactivity. The following preparations were administered in single doses.

Preparation A (for the i.v. and i.m. administration):

Glucosamine sulfate 400 mg (equivalent to 1.75 mmol DG), water for injection 2 ml. The radioactivity of the whole dose was 50 μ Ci.

Preparation B (for the oral administration):

Glucosamine sulfate 250 mg (equivalent to 1.10 mmol DG), water for injection 5 ml. The radioactivity of the whole dose was 50 μ Ci.

The preparations were administered in the morning at about 9 a.m. to the subjects fasting since 10 h. After 1 h a light breakfast was administered. The following meals were those usual for the subjects. The subjects were asked to abstain from smoking and alcoholic beverages from 24 h before administration of the preparations till the end of the sampling time.

2.3. Samples collection

Blood: Blood samples were taken from a cubital vein into heparinized tubes and immediately centrifuged at 4000 r.p.m. to obtain plasma. The blood samples were collected at the following times after administration.

After i.v. administration: 0 (immediately before administration), 0.083, 0.25, 0.5, 1, 2, 4, 6, 8, 10, 24, 48, 72, 96 and 120 h.

After i.m. and oral administration: 0 (immediately before administration), 0.5, 1, 2, 4, 6, 8, 10, 24, 48, 72, 96 and 120 h.

Urine: The total urine excreted during the following intervals after administration was collected: 0-4, 4-8, 8-24, 24-48, 48-72, 72-96 and 96-120 h. The volume of each fraction was measured and recorded. A portion of each fraction (about 30 ml) was transferred to appropriate containers and the remainder disposed off.

Feces: The feces excreted during the following intervals after administration were collected: 0-24, 24-48, 48-72, 72-96 and 96-120 h. The weight of each feces samples was measured and recorded.

2.4. Measurement of radioactivity

A Packard Tricarb 2000 CA counter was used. Sample quenching was monitored with an external ^{133}Ba standard source. The d.p.m. were obtained from the corresponding c.p.m. using the standard quench curve. Counting was performed for 20 min or up to a statistical confidence level of 2 sigma error equal or less than 5 %. The counting efficiency was 80-90 % for plasma and urine and 70-80 % for feces.

2.5. Sample preparation and analysis

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Whole plasma: A 0.2 ml aliquot of each plasma sample was introduced into a scintillation vial and counted after addition of 10 ml of Instagel (Packard Instruments, Milan, Italy) scintillation solution. All analyses were performed in duplicate.

Deproteinized plasma: A 0.25 ml aliquot of each plasma sample was deproteinized by adding the same volume of 10 % trichloroacetic acid and centrifuging at 3000 r.p.m. for 10 min. A 0.15 ml aliquot of the supernatant was introduced into a scintillation vial and counted after addition of 10 ml of Instagel scintillation solution. All analyses were performed in duplicate.

Urine: A 0.2-1.0 ml aliquot of each urine sample was introduced into a scintillation vial and counted after addition of 10 ml of Instagel scintillation solution. All analyses were performed in duplicate.

Feces: Each total feces sample was lyophilized and ground to a fine powder. A 200-300 mg aliquot was combusted in an oxidizer under oxygen, the combustion products were adsorbed on Carbosorb (Packard) collected into a scintillation vial and counted after addition of 10 ml of Hionic-Fluor (Packard) scintillation solution. All analyses were performed in duplicate.

2.6. Pharmacokinetic evaluation and statistical calculations

Calculated were AUC, by the trapezoid method, AUC, C_{max} , t_{max} , A_e , and dA_e/dt . Fitting with compartment models was made according to Wagner [9] and Ritschel [4].

2.7. Recording of adverse events

During the study and in the following month the subjects were questioned whether any adverse event had occurred.

2.8. Ethical provisions

The protocol was approved by the Institutional Ethics Committee of the University of Perugia, which also stated the maximum number of subjects, their sex (males only) and the maximum radioactivity dose allowed for this study. The informed consent to participate to the study was obtained by the investigators in writing from each subject. The study was made in compliance with the Good Clinical Practice in the European Community [1], and the rules set forth in the Helsinki Declaration [6]. The subjects were insured against any risk or damage deriving from the trial.

3. Results

3.1. Radioactivity in blood plasma

The radioactivity measured in whole plasma and in deproteinized plasma was converted into μmol equivalents per litre of DG. The concentrations found in deproteinized plasma were subtracted from the concentrations in whole plasma in order to obtain the concentrations incorporated in the plasma proteins.

The results found in deproteinized plasma are given in Table 2 and in Fig. 1. The results show the very high concentration of radioactivity in deproteinized plasma immediately after i.v. administration. This radioactivity, which originates from intact DG, disappears with a K_{el} of 23 h^{-1} . 30 min after administration the radioactivity is 1.4 % of that found 5 min after administration. Between 30 min and 6 h the radioactivity disappears with a K_{el} of 0.38 h^{-1} . During these periods, after i.m. administration, radioactivity in deproteinized plasma is present and disappears with a similar K_{el} . After oral administration no detectable radioactivity is found in deproteinized plasma.

The results regarding the radioactivity incorporated in the plasma proteins are given in Table 2 and Fig. 2. After i.v. the radioactivity appears after a lag time of 1–2 h after administration and then gradually increases to reach the peak between 6 and 24 h after administration. The radioactivity then slowly disappears, but measurable amounts of radioactivity are present in the plasma proteins even 120 h after administration. After i.m. administration the radioactivity found in plasma proteins shows patterns which are practically superimposable to those found after i.v. administration. After oral administration the radioactivity in plasma proteins appears with a lag time of 1–2 h and then follows patterns which are similar

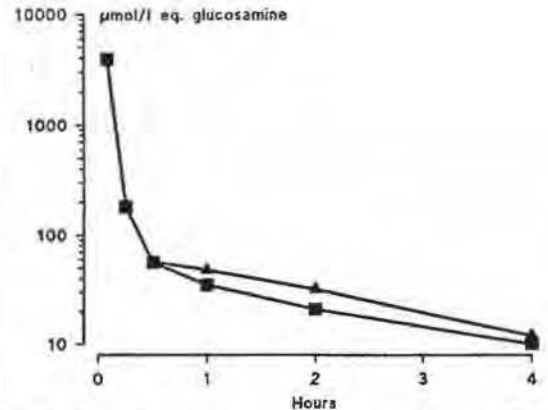


Fig. 1: Radioactivity in deproteinized plasma originating from ^{14}C -DG. Averages of 2 subjects each after i.v. (■) and i.m. (▲) administration.

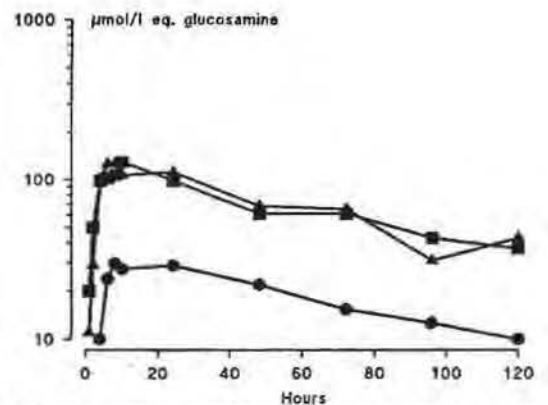


Fig. 2: Radioactivity in plasma proteins originating from ^{14}C -DG incorporated in plasma proteins. Averages of 2 subjects each after i.v. (■), i.m. (▲) and oral (●) administration.

to those found after i.v. or i.m. administration but with concentrations which are 3–4 times lower than those found after parenteral administration.

3.2. Radioactivity excreted in urine

The cumulated radioactivity excreted in urine and expressed as percent of the administered dose is given in Table 3 and in Fig. 3. The urinary excretion in the 120 h

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Table 2: Radioactivity expressed as $\mu\text{mol/l}$ equivalents of DG in deproteinized plasma (Dep. pl.) and in plasma proteins (Pl. prot.).

Hour	I.v. administration				I.m. administration				Oral administration			
	Dep. pl.		Pl. prot.		Dep. pl.		Pl. prot.		Dep. pl.		Pl. prot.	
	Subj. 1	Subj. 2	Subj. 1	Subj. 2	Subj. 3	Subj. 4	Subj. 3	Subj. 4	Subj. 5	Subj. 6	Subj. 5	Subj. 6
0	0	0	0	0	0	0	0	0	0.0	0.0	0.0	0.0
0.083	5748	2070	0	0	0	0	0	0	0.0	0.0	0.0	0.0
0.25	273	89	0	0	0	0	0	0	0.0	0.0	0.0	0.0
0.5	68	43	0	3	57	57	3	1	1.1	0.6	0.0	0.6
1	44	26	22	17	48	49	11	12	1.1	1.7	0.6	0.6
2	28	14	49	51	29	35	37	21	1.7	2.2	1.1	2.8
4	12	8	111	85	14	11	119	83	3.9	2.8	9.5	10.6
6	10	4	105	99	10	4	158	92	2.2	1.1	27.9	19.5
8	7	6	122	92	6	3	142	111	1.7	0.6	33.5	25.7
10	5	2	153	102	4	5	145	67	2.8	1.1	36.8	17.9
24	3	2	118	78	3	2	129	93	1.7	0.6	31.8	25.7
48	2	2	68	54	2	1	65	70	0.6	0.0	23.4	20.1
72	2	1	60	63	2	2	71	59	0.0	0.0	17.3	13.4
96	1	1	40	46	1	1	41	22	0.0	0.0	14.5	10.6
120	2	0	34	40	1	2	47	40	0.0	0.0	9.5	10.0

radioactivity disappears very rapidly, with an initial $t_{1/2} = 0.03$ h, followed by a slower rate of $t_{1/2} = 0.28$ h between 0.25–0.5 h after administration. After 4 h the radioactivity originating from DG is below the limits of the errors of the method. Starting from the 1st h after administration a radioactivity incorporated in the plasma proteins appears, reaching the peak of 128 $\mu\text{mol/l}$ equivalents DG 10 h after administration. Then this radioactivity declines, with a $t_{1/2}$ elimination time of 70 h. Detectable amounts of this radioactivity are present in the plasma proteins even 120 h after administration. The AUC of total radioactivity after i.v. administration is of 12.94 $\text{mmol/l} \times \text{h}$, and this represents the AUC of a 100 % bioavailable DG. Two confounding phenomena are however present, i.e. the elimination of radioactivity with $^{14}\text{CO}_2$ of the expired air and the urinary excretion of radioactivity, both depended on the concentration of DG in blood and tissues. These biasing factors must be considered when the AUC of radioactivities after i.v. administration is used as reference for the AUC after other administration routes for the evaluation of the relative bioavailabilities.

I.m. administration: After i.m. administration the initial very high plasma levels of radioactivity in deproteinized plasma found after i.v. administration are missing, but from 30 min after administration onwards, the pattern of radioactivity from deproteinized plasma and from plasma proteins are very similar to those found after i.v. administration. The bioavailability of DG after i.m. administration calculated from the AUC is of 96 % of that after i.v. administration.

Oral administration: After oral administration no radioactivity is found in deproteinized plasma. The radioactivity from plasma proteins shows patterns that are similar to those after i.v. or i.m. administration, but the levels are 3–5 times lower. The AUC of radioactivity after oral administration is 26 % of that after i.v. administration.

4.2. Urinary excretion of radioactivity

The pattern of the urinary excretion of radioactivity and the amounts excreted are shown in Fig. 3 and Table 3.

After i.v. administration the urinary excretion rate is very high in the first 4 h after administration, with a $t_{1/2}$ of 2 h. In the first 4 h 83 % of the radioactivity eliminated with the urine is recovered.

After i.m. administration the urinary excretion is greater than after i.v. administration, but the available data do not allow to estimate whether this difference is statistically or clinically significant. Also after i.m. administration the initial urinary excretion rate is fast, with a $t_{1/2}$ of 2 h, and in the 4 h following the administration 73 % of the totally excreted radioactivity is recovered.

After oral administration the urinary excretion is about 30 % of that after i.v. or i.m. administration averaged. The maximum excretion rate is achieved between 4 and 8 h, with a $t_{1/2}$ of about 3 h. 8 h after administration 77 % of the totally excreted radioactivity is recovered.

Table 6: Cumulated radioactivity recovered from urine and feces in percent of administered dose.

Hour	I.v. administ.	I.m. administ.	Oral administ.
24	24	27	3
48	26	35	13
72	27	36	20
96	28	37	21
120	28	37	21

4.3. Fecal excretion of radioactivity

After i.v. or i.m. administration the fecal excretion of radioactivity is very small, accounting for less than 1 % of the administered dose.

Conversely after oral administration the fecal excretion of radioactivity accounts for 11 % of the administered dose, and occurs mainly between 24 and 72 h after administration. After oral administration the radioactivity excreted with the feces represents probably the non-absorbed fraction of DG. It can therefore be estimated that GS after oral administration allows the gastrointestinal absorption of almost 90 % of the administered dose.

4.4. Balance of radioactivity

With the results of the urinary and fecal excretion it is possible to discuss the balance of the radioactivity of the administered GS by i.v., i.m. or oral route. The data are summarized in Table 6, which shows that the radioactivity recovered in urine and feces in 120 h after administration accounts for a relatively small fraction of the administered dose, i.e. 28 % after i.v., 37 % after i.m., and 21 % after oral administration. The unrecovered fraction is probably in small part retained in the tissues and in largest part metabolized to small endproducts and eliminated as $^{14}\text{CO}_2$, as previously found in rat and dog studies.

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Antiarthritic Effects of Glucosamine Sulfate Studied in Animal Models

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Summary

The antireactive activity of glucosamine sulfate (GS) (CAS 29031-19-4) was tested in the rat in experimental models of subacute inflammation (sponge granuloma and croton oil granuloma), on subacute mechanical arthritis (kaolin arthritis) and in immunological-reactive arthritis and generalized inflammation (adjuvant arthritis). On these models GS was found effective in oral daily doses of 50–800 mg/kg. The potency of GS in comparison of that of indometacin used in the same tests as reference substance was found 50–300 times lower. Since, however, the toxicity of indometacin in chronic toxicity experiments is 1000–4000 times larger, the therapeutic margin with regard to prolonged treatments of inflammatory disorders results 10–30 times more favourable for GS than for indometacin. GS can therefore be considered as a drug of choice for prolonged oral treatment of rheumatic disorders.

Zusammenfassung

Antiarthritische Wirkungen von Glucosaminsulfat im Tier-Modell

In experimentellen Modellen der subakuten Entzündung (Schwamm- und Krottonöl-Granulom), der subakuten mechanisch induzierten Arthritis (Kaolin-Arthritis) sowie der immunologisch-reaktiven Arthritis und generalisierten Entzündung (Adjuvant-Arthritis) wurde die antireaktive Aktivität von Glucosaminsulfat (GS) (CAS 29031-19-4) bei der Ratte überprüft. In diesen Modellen erwies sich GS bei oraler täglicher Dosis von 50–800 mg/kg KG als effektiv. Im Vergleich zur Wirkung von Indometacin, das in diesen Modellen als Referenz-Substanz eingesetzt worden war, war die Wirkung von GS 50–300mal geringer. Da jedoch die Toxizität von Indometacin in Untersuchungen zur chronischen Toxizität 1000–4000mal größer war, resultiert daraus hinsichtlich der Langzeittherapie von entzündlichen Krankheiten ein 10–30mal günstigerer therapeutischer Index von GS. GS sollte daher als Arzneimittel der Wahl in der oralen Langzeit-Therapie rheumatischer Krankheiten in Betracht gezogen werden.

Key words: Anti-inflammatories, non-steroidal · Arthritis, experimental · CAS 29031-19-4 · Glucosamine sulfate, chronic toxicity studies, pharmacology

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1. Introduction

Glucosamine (CAS 3416-24-8) is an aminomonosaccharide, naturally present in the body and especially in the articular cartilages, where it is chemically embodied in different types of glycosaminoglycans of the matrix of the cartilage. Its main physiological role is to stimulate the biosynthesis of proteoglycans [11], for which it represents also one of the essential substrates [21]. Glucosamine has also cyclooxygenase-independent antireactive properties [18, 22, 23] and was successfully introduced in the therapy of arthrosis [1, 3, 4, 5, 10, 14, 15, 19, 28]. Also recent clinical investigations support the disease-modifying properties of glucosamine in osteoarthritis [13, 17, 20]. The purpose of the present investigation is the study of the effects of glucosamine in subacute and chronic models of inflammation, particularly in models of experimental arthritis.

2. Materials and methods

2.1. Substances

For oral administration glucosamine sulfate (GS) (CAS 29031-19-4) was used, obtained from Rotta Research Laboratorium S.p.A., Monza (Italy). For parenteral administration was used the injectable solution Dona 200-S[®], obtained from Opfermann Arzneimittel GmbH, Wiehl (FRG). This solution contains in 1 ml 133 mg of GS.

Carrageenin was obtained from Gianni S.p.A., Milan (Italy).

Evans blue, Coomassie brilliant blue and carboxymethylcellulose (CMC) were obtained from Fluka, Buchs (Switzerland).

PGE₂, croton oil, kaolin and casein sodium were obtained from Sigma, St. Louis, MO (USA).

Benzalkonium chloride (Zefirol[®]) was obtained from Bayer, Milan (Italy).

Mycobacterium butyricum was obtained from Difco Laboratories, Detroit, MI (USA).

Bovine serum albumin was obtained from E. Merck, Darmstadt (FRG).

2.2. Animals

Sprague-Dawley rats were used, obtained from Charles River Laboratories (Calco, Italy). The rats were fed with Vogt-Müller diet (Dr. Piccioni, Brescia, Italy) and water ad libitum. The diet

Rotta Research Laboratorium S.p.A., Monza (Italy)

was controlled for chemical contaminants and complied with the limits of the Toxic Control Act E.P.A. (Fed. Reg. part 4, 26, 7, 1979). The animals were housed for at least 1 week prior use in the animal house of Rotta Research Laboratory at a controlled temperature of $22 \pm 2^\circ\text{C}$, humidity of $60 \pm 10\%$ and 12-h photocycles.

2.3. Models of subacute inflammation and experimental arthritis

In the following studies the animals were randomly assigned to the control and treatment groups. For parenteral administration the substances under investigation were dissolved in an aqueous solution of 0.9% NaCl and administered at constant volume. For oral administration the substances were suspended in aqueous 1.0% CMC and administered at constant volume with a stomach tube. The control animals received the vehicle only.

2.3.1. Sponge granuloma in the rat

Male rats of 150 g body weight were used. Sponges for implantation were prepared from polyvinyl foam sheets of 5 mm thickness [9]. Prior to the implantation the sponges were soaked in 1% carrageenin. Under light ether anaesthesia a 20 mm dorsal transversal incision was made in the skin and the dermis separated from the underlying muscles by inserting a blunt forceps to form two cavities in which 2 sponges per rat were inserted. The dorsal incision was closed with metallic clips. The substances under investigation were administered orally, b.i.d. during 5 days. The sponges were removed from the anesthetized rat by opening the incision 5 days after the implantation. Immediately after removal the right sponge was placed in a 2 ml syringe and the exudate squeezed out by piston pressure and centrifuged at 800 rpm for 10 min. The exudate was used for the determination of the prostaglandine-like activity, the content of proteins and the number of leukocytes.

The prostaglandine-like activity was assayed on gastric fundus preparations [27], using the laminar flow technique [7] on strips of gastric fundus of male rats of 200 g body weight. The rats were sacrificed by cervical dislocation and exsanguination. The abdomen was opened, the stomach was isolated and the fundal part was dissected and transferred to a Petri dish containing Krebs solution. The fundal end was opened longitudinally and a strip was prepared making alternate transverse cuts on opposite sides of the muscle. The preparation was immersed in mineral oil thermostated at 37°C . Oxygenated (5% CO_2 in O_2) Krebs solution was delivered directly over the serosal surface of the tissue through a small polyethylene tube. The rate of superfusion was 0.3 ml/min. The specificity of the bioassay was increased adding to the Krebs solution pharmacological antagonists at the following concentrations ($\mu\text{g/ml}$): propranolol 2.28, mepyramine 0.14, phenoxybenzamine 0.15, atropine 0.19, methysergide 0.20. Indometacin 1 $\mu\text{g/ml}$ was also added to prevent the endogenous synthesis of prostaglandine-like substances. The strip was connected to an isotonic transducer, applying a resting tension of 2 g. After an equilibration period of 2 h the strip was stimulated by increasing doses from 0.1 to 1 ng/ml of PGE_2 to obtain a dose-response curve. The sample was administered in a volume between 20 and 50 μl and the contractions of the strip were compared with the contractions obtained with different concentrations of PGE_2 .

The protein content was determined using Coomassie Brilliant Blue [2].

2.3.2. Granuloma provoked by croton oil in the rat

Used were male rats of 150 g body weight. Granulomas were provoked injecting 0.5 ml of a sterile 1% solution of croton oil into a pouch made in the dorsal thoracic region of the rat by a S.C. injection of 25 ml of air [8]. After 7 days the animals were sacrificed under ether anaesthesia, the exudate and granuloma tissue were collected and weighed, and the weights were expressed in mg/kg body weight. The substances under investigation were administered b.i.d. i.p. or orally by gavage. The first administration was made 1 h before the preparation of the granuloma pouch.

2.3.3. Arthritis provoked by kaolin in the tibio-tarsal articulation of the rat

Uses were male rats of 125–150 g body weight. Arthritis was provoked by an injection in the tibio-tarsal articulation of 0.2 ml of a 10% suspension of kaolin in sterile saline. The degree of

arthritis was estimated by the volume of the paw. At the 5th day, 1 ml/kg of a 1% solution of Evans blue was injected i.v. After 2 h the rats were sacrificed under ether anaesthesia, the tibio-tarsal articulation removed and digested in 3 ml of concentrated HCl at 37°C for 18 h. At the end of the digestion 4 ml of a 12.8% benzalkonium chloride solution were added to the samples and the mixture was shaken for 30 min. Benzalkonium complexes with Evans blue and this complex is selectively soluble in chloroform. To each sample 7 ml of chloroform were added and strongly shaken. After a few minutes the chloroform layer with the Evans blue complex was collected, filtered through a silicon filter (1 PS Whatman, England) and brought to a final volume of 10 ml. The concentration of Evans blue was photometrically measured at 620 nm against a chloroform blank. The results were expressed as μg of Evans blue per mg of weight of the tibio-tarsal articulation. The substances under investigation were administered orally 1 h before the injection of kaolin and then twice daily for the whole duration of the experiment.

2.3.4. Adjuvant arthritis in the rat

Used were male rats with 125–175 g body weight. The arthritic syndrome was induced by an intradermal injection into the subplantar region of the right hind paw of 0.5 mg Mycobacterium butyricum in 0.1 ml of white mineral oil [29]. The substances under investigation were administered orally once a day for 21 consecutive days, beginning shortly before the injection of adjuvant. Arthritis develops after 7–21 days and causes swelling of the injected paw (primary lesion), decrease of body weight, swelling of the non injected paws, and nodules on the ears and tail (secondary lesions). The assessed parameters were:

- incidence of arthritis, i.e. the percentage of animals with nodules,
- body weight, recorded daily,
- volume of the right and left hind paw, recorded at weekly intervals,
- numbers of nodules on the ears, paws or tail, recorded at the 21th day.

2.4. Statistical calculations

Arithmetic averages, standard deviations and standard errors were calculated by the conventional methods. Regression lines and related constants were calculated by the least squares method.

The percent protective effect (P) for each animal, at each dose of the tested drugs and at each testing time was calculated as:

$$P = 100 \times (\text{CG} - \text{DG}) / \text{CG}$$

where "CG" is the average of the percent variation of investigated variable of each animal with respect to the initial value prior treatment and "DG" is the percent variation of the investigated variable of each animal of the treatment group with respect to the initial value. The Ps were plotted against the logarithm of the doses of the substance under investigation and the ED_{50} , i.e. the dose effective in giving an xx% protection, and their $p = 0.05$ fiducial limits were calculated from the regression line. In the studies with two control groups, e.g. with "arthritis" and "normal" animals, the percent protective effect was calculated for each animal as:

$$P = 100 \times (T - U) / (C - U)$$

where "T" is the measurement of the variable in the treated group, "U" is the average of the variable in the group treated with the proinflammatory agent but not with the substance under investigation, and "C" is the average of the variable in the group not treated with the proinflammatory agent and not treated with the substance under investigation. The ED_{50} were calculated as above described.

3. Results

3.1. Protection against the subacute inflammatory effects provoked by subcutaneous implants of polyvinyl sponges in the rat

The results are given in Table 1, with the doses of GS and of indometacin which inhibited by 30% (ED_{30}) the subacute inflammatory effects provoked by the subcutaneous implant of a polyvinyl sponge soaked with 1% carrageenin.

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Table 1: Protective effects of GS or indometacin orally administered on the inflammatory effects of a polyvinyl sponge subcutaneously implanted. The results were obtained on 10 rats per dose and 3 doses were administered. The ED₃₀ and their $p = 0.05$ fiducial limits (in brackets) are given.

Effects on exudate	ED ₃₀ (mg/kg)		Relative potency
	GS	Indometacin	
Volume	570 (208–1560)	1.9 (0.7–4.9)	0.003
No. of leucocytes	418 (320–546)	2.4 (1.3–4.6)	0.006
Proteins contents	611 (171–2188)	2.6 (1.1–6.0)	0.004
PGE ₂ content	Inactive	0.9 (0.3–2.5)	ND

ND = not determinable.

Table 2: Protective effects of GS or indometacin i.p. administered on the inflammatory effects of croton oil granuloma. The results were obtained on 10 rats per dose and 3 doses were administered. The ED₃₀ and their $p = 0.05$ fiducial limits (in brackets) are given.

Effects	ED ₃₀ (mg/kg)		Relative potency
	GS	Indometacin	
Volume of exudate	664 (238–1849)	2.1 (1.5–2.9)	0.003
Weight of granuloma tissue	631 (149–2679)	3.6 (0.7–18)	0.006

3.2. Protection against the subacute inflammatory effects provoked by croton oil in a subcutaneous pouch in the rat

The results are given in Table 2 with the doses of GS and of indometacin which inhibited by 30 % (ED₃₀) the subacute inflammatory effects provoked by the injection of croton oil in a subcutaneous pouch in the rat.

3.3. Protection against kaolin arthritis in the rat

The results are given in Table 3. The doses of GS and of indometacin are given which inhibited by 30 % (ED₃₀) the subacute inflammatory effects provoked by an injection of kaolin in the tibio-tarsal articulation of the rat.

3.4. Protection against adjuvant arthritis in the rat

The results are given in Table 4. The doses of GS and of indometacin are given which inhibited by 30 % (ED₃₀) the chronic inflammatory effects provoked by the subplantar injection of adjuvant in the rat.

4. Discussion

Glucosamine sulfate (GS) given orally or parenterally is used in human therapy for prolonged treatment of osteoarthritis and other rheumatic disorders. Pharmacodynamic studies on the possible mechanism of action which could be the rationale for the efficacy of GS showed that GS has cyclooxygenase-independent antireactive properties and no analgesic effects [23]. The antireactive and antiarthritic effects of GS were confirmed in the present study on experimental models of subacute inflammatory processes and of arthritis. Actually GS was found effective against the subacute exudative-granulomatous inflammation due to subcutaneous implants of carrageenin-soaked polyvinyl sponges and against the croton oil elicited subcutaneous granuloma in rats. GS was also found effective in a model of mechanical-induced arthritis,

Table 3: Protective effects of GS or indometacin orally administered on the articular inflammatory effects provoked by kaolin. The results were obtained on 10 rats per dose and 3 doses were administered. The ED₃₀ and their $p = 0.05$ fiducial limits (in brackets) are given.

Effects	ED ₃₀ (mg/kg)		Relative potency
	GS	Indometacin	
Evans blue diffusion	531 (272–1038)	2.9 (1.5–6)	0.003
Volume of the injected paw	833 (513–1521)	8.4 (2.5–29)	0.01

Table 4: Protective effects of GS or indometacin orally administered against symptoms of adjuvant arthritis. The results were obtained on 12 rats per dose and 3 doses were administered. The ED₃₀ and their $p = 0.05$ fiducial limits (in brackets) are given.

Effects	ED ₃₀ (mg/kg)		Relative potency
	GS	Indometacin	
Incidence of arthritis	184 (76–446)	0.9 (0.3–3)	0.005
Decrease of body weight	455 (248–836)	1.6 (0.6–5)	0.004
Volume of injected paw	229 (148–353)	1.5 (0.5–5)	0.007
Volume of not injected paw	111 (63–194)	1.9 (0.6–6)	0.02
Number of nodules	49 (3–855)	1.1 (0.4–3)	0.02

tis, i.e. against the kaolin-elicited arthritis in the tibio-tarsal articulation in the rat. Finally GS was found effective against the adjuvant arthritis in the rat, which has an immunological-reactive pathogenesis and is considered the best model for human rheumatoid arthritis and osteoarthritis. The efficacy of GS on all these models confirm the antireactive activity of GS in subacute or chronic inflammation and arthritis, and may represent the rationale of the human therapeutic use of GS in chronic rheumatic disorders.

In the experiments reported in this publication GS was always tested using indometacin as reference agent. In potency indometacin was 50–300 times superior to GS. For a therapeutic use, especially in the case of prolonged treatments, potency alone is however less relevant, and the most important parameter is the therapeutic margin, i.e. the ratio between the effective and the toxic doses in repeated administrations. For indometacin the highest safe daily oral doses in chronic toxicity tests are less than 1 mg/kg, both in rats and in dogs (Table 5). The most sensitive target is the GI tract, where indometacin provokes erosions, hemorrhages and ulcers in the small intestine. Conversely daily oral doses of GS up to 2700 mg/kg in the rat and 2149 mg/kg in the dog in 1-year and respectively 6-month experiments did not provoke anatomical lesions of the GI tract or of other organs (Table 5).

In conclusion the potency of GS in models of subacute inflammation and chronic arthritis is 50–300 times lower than that of indometacin, but the safe doses of GS in chronic administration are at least 1000–4000 times larger than those of indometacin. The therapeutic margin of a prolonged use of GS is therefore 10–30 times larger than that of indometacin. Taking into account the antireactive properties of GS [23], the bioavailability of GS after oral administration [24, 25], the large therapeutic margin in experimental subacute and chronic inflammatory and arthritic models, and the safety in prolonged oral administration it is justified to classify GS as a potential disease-modifying drug for long-term treatments of osteoarthritis and other rheumatic disorders.

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Table 5: Repeated doses toxicity of indometacin (Indo) and of GS by oral administration.

Substance	Species	Daily doses (mg/kg)	Duration (weeks)	Safe dose ^{a)} (mg/kg)	Ref.
Indo	rat	0.1, 0.25, 0.5, 0.75, 1.6, 5.0 ^{b)}	35	0.75	[26]
Indo	dog	2, 5, 10, 20 ^{c)}	7	< 2	[26]
Indo	dog	0.25, 0.5, 1.0 ^{d)}	129	0.5	[26]
GS	rat	300, 900, 2700 ^{e)}	52	2700	[12]
GS	dog	159, 478, 2149 ^{f)}	26	2149	[16]

^{a)} Daily dose which did not influence body growth and did not provoke drug-related anatomical or histopathological lesion.

^{b)} Daily doses of 1.6 and of 5.0 mg/kg provoked anorexia, body weight depression, diarrhea, blood in stools. In the jejunum erosions, ulcers and perforations were found.

^{c)} Daily doses of 2 mg/kg produced anorexia, diarrhea and blood in stools. In the ileum ulcers were found, with dose-depending severity up to perforation.

^{d)} Daily doses of 1 mg/kg produced occasional blood in stools.

^{e)} The daily dose of 2700 mg/kg provoked 7 premature death in the group of 60 animals. The difference vs the controls (2/60) is not statistically significant (Fisher exact probability = 0.16). No drug related macroscopic or histopathological lesions were found in the deceased animals or in the survivors.

^{f)} Even at the highest daily dose of 2149 mg/kg no clinical or laboratory or histopathological toxic sign could be detected.

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Effects of oral glucosamine and chondroitin sulfate alone and in combination on the metabolism of SHR and SD rats

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Abstract

Glucosamine (G), often combined with chondroitin sulfate (CS), is a popular natural supplement used widely to treat osteoarthritis. However, use of glucosamine has been linked to development of insulin resistance. To assess the association between glucosamine and insulin resistance more closely, we challenged two rat strains highly sensitive to sugar-induced insulin resistance—Sprague-Dawley (SD) and Spontaneously Hypertensive (SHR) rats. Since elevations of systolic blood pressure (SBP) have been found to be an early and highly sensitive sign of insulin resistance in these two rat strains, we used this parameter as our primary endpoint. Four groups of both rat strains received either no agent (control), G, CS, or a combination of both for 9 weeks. The intake of each agent was calculated to be approximately 3–7 times comparable to human dose. Throughout the study, SBP of both strains consuming the two ingredients alone and in combination were not elevated. Rather, they were significantly lower than control, contrary to what is found in glucose-induced insulin resistance in rats. Over the study period, body weights of the four groups of SD and SHR did not vary significantly. Furthermore, no consistent trends in circulating glucose concentrations were found among the four different groups in the two strains after oral challenge with glucose. Finally, no significant histological differences were found in hearts, kidneys, and livers among the various groups of SHR and SD. From the above result, we conclude that glucosamine and chondroitin sulfate given alone or together do not produce insulin resistance or other related perturbations in two rat strains highly sensitive to sugar-induced insulin resistance. (*Mol Cell Biochem* 225: 85–91, 2001)

Key words: glucosamine insulin resistance, chondroitin sulfate insulin resistance, insulin resistance glucosamine, insulin resistance chondroitin sulfate, glucosamine toxicity, toxicity glucosamine

Introduction

The public has recently recognized the therapeutic potential of two natural products for treating osteoarthritis—glucosamine (G) and chondroitin sulfate (CS) [1, 2]. Numerous studies, many double-blinded and placebo-controlled, support the benefit of these agents [3–11], as does a recent meta-analysis [12]. Unfortunately, other reports suggest that use

of supplemental G may contribute to insulin resistance in those prone to develop this condition, and may even cause diabetes mellitus [13, 14]. That G has the potential to interfere with glucose/insulin metabolism has long been recognized through both *in vitro* and *in vivo* findings [15–22]. The ability of elevated circulating glucose concentrations to cause insulin resistance has been linked to excess flux through the hexosamine biosynthetic pathway [16]. The hexosamine

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pathway normally accounts for approximately 2% of total cellular glucose flux [17], but an increased flux has been postulated to blunt insulin-stimulated glucose uptake and glycogen synthesis. It also down-regulates glucose transporters in sensitive tissues, and augments synthesis of fatty acids and triglycerides [22].

While it is generally recognized that G infusions in high loads can produce insulin resistance in normoglycemic rats [19], a question arises whether resistance could occur following oral ingestion in a dose-range relevant to therapeutic dose. As a first approximation, we assessed the association between oral G intake and diabetes by examining selected markers of insulin sensitivity in two rat strains highly responsive to sugar-induced insulin resistance, namely Spontaneously Hypertensive (SHR) and Sprague-Dawley (SD) rats. We also examined these same effects for CS, which is commonly added to G for the management of osteoarthritis [1, 2].

Materials and methods

The protocol for the entire study was approved by the Animal Welfare Board at Georgetown University, USA. Thirty-two male Spontaneously Hypertensive Rats (SHR) and 32 male Sprague-Dawley (SD) rats, weighing 200–300 g were obtained from Harlan, Indianapolis, IN, USA. Rats from each strain were grouped according to the dietary regimen received (Table 1). Eight SD and SHR were given either baseline diet (BD), BD + G.HCl (supplied by Rexall/Sundown, Boca Raton, FL, USA) 0.5% w/w, or BD + CS (supplied by Rexall/Sundown, Boca Raton, FL, USA) 0.4% w/w, or BD + the combination of both natural substances at the same concentrations for 9 weeks. Approximately 9 weeks (or 2 months) exposure is necessary to bring about insulin resistance in rats,

and accordingly, this time period was selected in this study to assess the effect of G and/or CS. A concentration of 0.5% w/w of G roughly calculates to 10–20 times the human dose of 1500 mg/day for a 70 kg human. This calculation is based upon the weight of the rat and their average food consumption. However, one study suggests that the dose also should be adjusted to the metabolic characteristics of the rat [23], which would bring the rat dose comparable to the human dose in the range of 3–7 times. This calculation would hold true for CS (0.4% w/w) which has an average recommended daily dose for humans 80% that of G, 1200 mg/day. Body weight (BW) and SBP were measured weekly. At the end of the study, bloods were drawn prior to sacrifice, and after sacrifice tissues were obtained for histological examination. Specific procedures were performed as follows:

Systolic Blood Pressure (SBP)

SBP was estimated by tail plethysmography in unanesthetized rats after warming [24]. Readings were taken 0.5–1 min apart. To be accepted, SBP measurements had to be stable for at least three consecutive readings.

Blood chemistries

Blood was drawn from the heart of anesthetized rats prior to termination of study. Rats were sacrificed following carbon dioxide euthanasia. Chemical analyses were performed by routine laboratory procedures. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and blood urea nitrogen (BUN) were measured using SIGMA Diagnostic Kits 51-UV, 52-UV and 67-UV, respectively. ALT is a biomarker of hepatotoxicity, while BUN is a biomarker of kidney injury. Damage or disease to the heart, liver, skeletal muscle, kidney and erythrocytes tissue such as myocardial infarction, viral hepatitis, liver necrosis, cirrhosis and muscular dystrophy may result in raised serum levels of AST.

Histology

Tissues were collected after euthanasia, and histological evaluation was performed by a commercial laboratory (Experimental Pathology Laboratories, Inc., Herndon, VA, USA) on specimens preserved in 10% formalin. Hematoxylin and eosin stained sections were prepared and examined microscopically on the kidneys, livers, and hearts of all rats by one of the authors (KAF). The examiner was blinded as to the group source from which the tissue was derived. The code was not broken until the last results were recorded.

Table 1. Composition of the basic diet

Ingredients	% by weight	% of calories
Starch and/or sucrose	57.00	52.1
Vegetable oil	16.44	36.0
Casein	13.00	11.9
Mineral mix, AIN 76A	4.00	
Vitamin mix, AIN 76A	1.20	
Cholesterol	1.10	
NaCl	0.50	
Choline bitartrate	0.50	
DL-Methionine	0.20	
Sodium cholate	0.02	
Ethoxyquin	0.04	
Cellulose	6.00*	

The basic diet for the control group is shown. *In the second through fourth diets, glucosamine 0.5% w/w, chondroitin sulfate 0.4% w/w, or the combination at the same concentrations replaced that portion of cellulose.

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Statistical analyses

Results are presented as mean \pm S.E.M. The majority of statistics were performed by one way analysis of variance (ANOVA) using repeated measures. SBP and BW were examined by two-way analyses of variance (one factor being diet and the second factor being time of examination). Where a significant effect of diet was detected by ANOVA ($p < 0.05$), the Dunnett's t-test was used to establish which differences between means reached statistical significance ($p < 0.05$) [25].

Results*Body weights*

Throughout the 9 weeks of study, the body weights among the four dietary groups of SD and the smaller SHR did not vary significantly. At the initiation of study, average body weights of each group within strains were virtually the same. Nine weeks later, the differences in final body weights \pm S.E.M. were not statistically significant: SD control = 518 ± 6.9 g, G = 524 ± 9.9 g, CS = 539 ± 8.7 g, and combination = 526 ± 12.8 g; SHR control = 385 ± 5.5 g, G = 385 ± 8.8 g, CS = 370 ± 8.5 g, and combination = 365 ± 10.1 g. (Figs 1 and 2).

Systolic Blood Pressure (SBP)

By the fourth week in SD and the third week in SHR animals, the SBP of the rats consuming the two ingredients and the

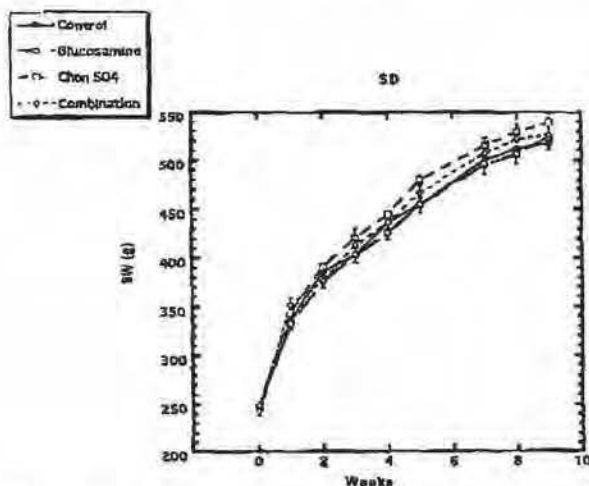


Fig. 1. Weight gain of Sprague-Dawley rats (SD) over the course of study. Mean \pm S.E.M. are shown.

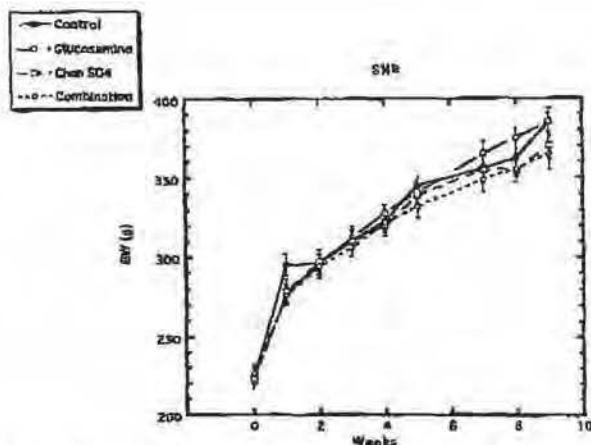


Fig. 2. Weight gain of Spontaneously Hypertensive rats (SHR) over the course of study. Mean \pm S.E.M. are shown.

combination were significantly lower than control. In SD, but not SHR, the combination tended to have a greater lowering effect on SBP than the individual components. In SHR, the SBP lowering effect of the natural products tended to become less at the 9 week reading (Figs 3 and 4).

Blood chemistries of SD and SHR among groups at end of 9 weeks

In SD, there were no relevant trends in the blood chemistries among the four groups. Although the BUN was significantly

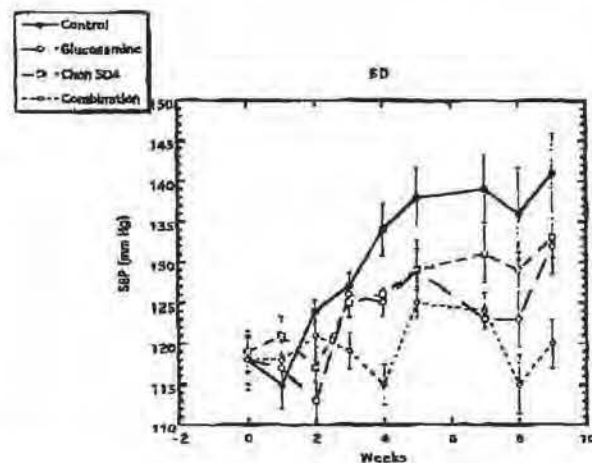


Fig. 3. SBP of Sprague-Dawley rats (SD) over the course of study. Mean \pm S.E.M. are shown.

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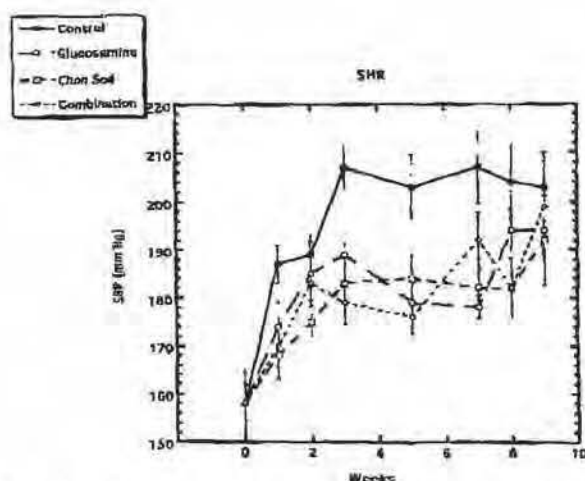


Fig. 4. SBP of Spontaneously Hypertensive rats (SHR) over the course of study. Mean \pm S.E.M. are shown.

lower in the control group compared to the other three groups, the circulating creatinine levels were not, suggesting that enhanced urea production played more of a role than renal perturbations in the differences. It is not clear why the triglycerides in the CS group tended to be lower than those of the rats in the G and combination groups, nor why the HDL levels were higher in the combination group compared to the control and CS groups.

In SHR, none of the blood chemistries showed statistically significant differences among groups. There was a tendency to have a higher triglyceride level among the CS group in contrast to the finding in the SD where the CS group showed the lowest value (Table 2).

Table 2. Blood chemistries at the end of 9 weeks in rats

Group	ALT units/L	AST units/L	BUN mg/dl	CRE mg/dl	CHOL mg/dl	TRIGLYC mg/dl	HDL mg/dl
Sprague-Dawley Rats							
Control	36 \pm 6.6	150 \pm 42	6.6 \pm 0.5	0.5 \pm 0.03	89 \pm 9.4	120 \pm 36	21 \pm 1.9
Glucosamine	44 \pm 7.3	136 \pm 36	8.3 \pm 0.5	0.6 \pm 0.04	109 \pm 18.4	190 \pm 69	25 \pm 2.9
Chon SO ₄	52 \pm 10.0	150 \pm 26	8.0 \pm 0.5	0.7 \pm 0.04	102 \pm 9.6	85 \pm 9.9	22 \pm 1.6
Combination	84 \pm 25.4	322 \pm 122	8.6 \pm 0.3	0.6 \pm 0.03	126 \pm 19	205 \pm 53	28 \pm 2.7
ANOVA p	> 0.14	> 0.22	< 0.03	> 0.07	> 0.10	< 0.04	< 0.05
Spontaneously Hypertensive Rats							
Control	95.3 \pm 21.6	324 \pm 70.6	15.0 \pm 3.1	0.47 \pm 0.07	61.5 \pm 5.1	112.3 \pm 10.7	20.6 \pm 1.2
Glucosamine	132 \pm 43.9	305 \pm 86	17.3 \pm 1.9	0.40 \pm 0.04	74.3 \pm 9.0	156.3 \pm 33.2	20.0 \pm 1.1
Chon SO ₄	107 \pm 25.1	265 \pm 37	18.3 \pm 3.5	0.43 \pm 0.07	64.6 \pm 7.0	217.3 \pm 55.6	23.3 \pm 1.6
Combination	132 \pm 55.2	394 \pm 101	16.0 \pm 2.9	0.40 \pm 0.07	66.7 \pm 5.2	163 \pm 52.3	22.6 \pm 1.4
ANOVA	> 0.86	> 0.68	> 0.82	> 0.83	> 0.35	> 0.07	> 0.30

Mean \pm S.E.M. are shown for 7-8 rats. Differences below data on SD rats indicate which rows show statistical significant from each other by the Dunnett's t-test. CRE - creatinine; CHOL - cholesterol; TRIGLY - triglycerides.

Glucose challenge

There were no statistically significant differences in glucose values after glucose challenge in either the SD or SHR after 9 weeks on the four regimens (Table 3).

Histopathological examinations

No significant differences in pathological observations were seen among the various groups of SHR and SD when examining hearts, kidneys, and livers. The majority of SHR had cardiomyopathy characterized by clusters of mononuclear cells with vacuolated cytoplasm. Fewer SD had cardiomyopathy although typical lesions of cardiomyopathy were observed in at least one SD rat from every group, even the control group. The SD rats, including the control group, had more animals with background renal changes such as tubular casts and tubular regeneration, typical of chronic progressive nephropathy, than most of the SHR. On the other hand, SHR had more background mononuclear cell infiltrates within the renal parenchyma or around the renal pelvis than SD rats. All livers of SD and SHR exhibited mild to moderately severe hepatocyte cytoplasmic vacuolization with a predominately portal distribution.

Discussion

For chronic osteoarthritis, much publicity surrounds the therapeutic benefits of oral supplementation with G alone or combined with CS [1, 2, 26-28]. Unlike many other natural supplements purported to have therapeutic benefits, the basis for their use derives from many good clinical trials [3-

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Table 3. Glucose tolerance test - Glucose (mg/dl)

Group	Base	30 min	60 min	120 min	240 min
Sprague-Dawley Rats					
Control	85.6 ± 3.2	113.8 ± 7.5	111.4 ± 7.7	124.2 ± 19.5	128.2 ± 16.5
Glucosamine	93.4 ± 7.3	145.8 ± 32.8	129.0 ± 16.0	104.8 ± 8.2	137.8 ± 16.1
Chondroitin	85.6 ± 8.0	109.2 ± 5.4	122.4 ± 8.0	126.2 ± 9.2	137.8 ± 6.9
Combination	83.0 ± 3.3	110.2 ± 11.5	114.8 ± 14.2	108.6 ± 4.9	102.8 ± 6.1
ANOVA p	< 0.48	< 0.49	< 0.74	< 0.47	< 0.17
Spontaneously Hypertensive Rats					
Control	64.6 ± 2.7	122.4 ± 13.9	100.6 ± 11.5	107.0 ± 15.9	110.2 ± 12.7
Glucosamine	59.2 ± 1.5	107.0 ± 17.3	91.0 ± 6.9	126.2 ± 7.9	126.3 ± 5.9
Chondroitin	61.8 ± 3.7	135.2 ± 31.6	143.0 ± 29.6	149.8 ± 26.2	152.4 ± 26.4
Combination	62.5 ± 2.3	150.7 ± 41.4	102.1 ± 8.8	94.3 ± 11.6	120.8 ± 28.0
ANOVA p	< 0.44	< 0.51	< 0.21	< 0.14	< 0.32

Mean ± S.E.M. of 5 rats.

12]. Their acceptance for therapy is becoming more commonplace based upon their potential to improve collagen metabolism and lack of severe gastrointestinal side effects possessed by many pharmaceutical analgesics used to relieve the pain of osteoarthritis. Because of their widespread use, it is important to examine any significant potential for adverse events, which could arise from the use of G and CS as therapeutic tools. One such possible adverse event associated with their use, at least with G, is the development of insulin resistance and even type two diabetes mellitus [13, 14].

Insulin resistance, which often occurs in the hyperglycemic state (glucose toxicity), has been attributed to excess flux of glucose breakdown products through the hexosamine biosynthetic pathway [15-22]. Although infusions of G into rats can induce insulin resistance [19], it is not known whether the oral intake of a reasonable amount of G, nearly equivalent to that recommended for the arthritic conditions, can also do so [29-31].

We initiated our examination of this question by assessing the influences of the oral ingestion of G hydrochloride and CS on insulin sensitivity in two strains of rats, normotensive Sprague Dawley (SD) and hypertensive Spontaneously Hypertensive Rats (SHR). These rats were chosen, because they show a strong proclivity to develop significant insulin resistance when challenged with oral sugars such as sucrose and fructose [29-31]. This phenomenon even occurs

when these strains are given sucrose in amounts similar to human intake [32]. Accordingly, if oral sugar ingestion produces this effect via stimulation of the hexosamine pathway, then what will the oral ingestion of G do? Based on the metabolism of rats and their body weight, we challenged the rats with a calculated dose that would be comparable to a human dose 3-7 times that recommended. As a first approximation, we believed this to be a reasonable challenge.

Over the 9 weeks of assessment, the two rat strains, when fed with the natural ingredients, did not lose weight, which we interpret as an excellent sign for lack of overall toxicity. At the initiation of the study, basic differences in blood chemistries existed between the normotensive SD and the hypertensive SHR. Comparing data from the SHR to the SD in the control groups at the end of the nine week study, circulating glucose and cholesterol were significantly lower, while circulating ALT, AST and BUN were significantly higher (Table 4).

Examining more specifically for the insulin resistant state, SHR and SD did not show increases in systolic blood pressure (SBP) following ingestion of these natural substances alone and in combination. In our hands, elevations of SBP have been the earliest and most consistent sign of insulin resistance in rats [33, 34]. Significant elevations in SBP occur long before changes in glucose/insulin and lipid parameters [34], and take place within days of sugar challenge

Table 4. Comparison of baseline blood chemistries of controls between SD and SHR

Rat	Glucose mg/dl	Insulin ng/ml	ALT unit/L	AST unit/L	BUN mg/dl	CRE mg/dl	CHOL mg/dl	TRYGLYC mg/dl	HDL mg/dl
SD	85.6 ± 3.2	0.19 ± .06	36 ± 7	150 ± 42	6.6 ± 0.5	0.5 ± 0.03	89 ± 9	120 ± 36	21 ± 2
SHR	64.6 ± 2.7	0.24 ± .06	95 ± 22	324 ± 71	15.0 ± 3.1	0.5 ± 0.1	62 ± 5	112 ± 11	21 ± 1
p	< 0.001	> 0.2	< 0.02	< 0.05	< 0.01	> 0.3	< 0.02	> 0.9	> 0.9

Mean ± S.E.M. are shown for 5-8 rats. Statistically significant differences between SD and SHR for glucose, ALT, AST, BUN, and cholesterol.

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depending on the breadth of challenge [29]. Important to our hypothesis, these 'sugar-induced' SBP elevations can be overcome by the concomitant administration of chromium [35-37]. The only generally recognized influence of chromium supplementation is to overcome insulin resistance [38-42]. G and/or CS did not increase SBP which would have been characteristic of sugar-induced insulin resistance. In fact, the two natural ingredients significantly lowered the SBP during their usage. It is not clear why this lowering occurred, and we are not aware of any similar reported effect in humans.

Examination of blood chemistries and histology also failed to indicate the development of insulin resistance or pathology consistent with this condition. Considering the four differing dietary groups, neither SD nor SHR showed any consistent trends in blood chemistries. Also, there were no statistically significant differences compared to controls in circulating glucose values after glucose challenge in either SD or SHR after nine weeks on the three test regimens. Finally, no significant histological differences were found in hearts, kidneys, and livers among the various groups of SHR and SD.

To summarize, the lack of an elevation in SBP and consistent effects on circulating glucose and insulin levels among the groups of the two rat strains by oral ingestion of G and CS suggest little effect on insulin sensitivity by these two natural substances when given orally at doses reasonable for therapy. Further, the lack of consistent effects on the other blood chemical parameters and the organ histology suggest no overall toxicity as well, at least under the conditions of the experiment. Therefore, we could not implicate either agent given alone or together in the development of insulin resistance or other toxicities in two rat strains highly sensitive to the glucose-induction of insulin resistance.

Acknowledgement

This study was financially supported by a grant from Rexall/Sundown, Inc., Boca Raton, FL, USA.

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Meeting of Cargill and CFSAN
22 January 2004

Participants and Agenda

Participants

Brent Rogers- Cargill Acidulants
John Bohlmann- Cargill Acidulants

James W. Anderson, M.D.- Professor of Medicine & Clinical Nutrition-
University of Kentucky
Joseph F. Borzelleca- Professor, Pharm & Tox, Medical College of Virginia
Robert J. Nicolosi- Professor and Director, Center for Health Sciences- University
of Massachusetts- Lowell

Agenda

Introductory Comments
Cargill's Glucosamine

Glucosamine- Safety and Efficacy
Safety
Animal data- ADME, toxicity
Human data- endogenous formation, metabolism, clinical studies
Efficacy
Osteoarthritis
Summary and Conclusions

GRAS status of Cargill's Glucosamine
Conclusions of Expert Panel: GRAS by scientific procedures- human data
supported by animal data

Guidance from the FDA

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Memorandum

Date: January 22, 2004
From: Karin Ricker
Subject: Product Under Development
To: File

Participants:

Visitors

Brent Rogers	Cargill Acidulants
John Bohlmann	Cargill Acidulants
Joseph Borzelleca	Medical College of Virginia
James W. Anderson	University of Kentucky
Robert Nicolosi	University of Massachusetts-Lowell

FDA

Mike DiNovi	HFS-255
Alison Edwards	HFS-255
Feleke Eshete	HFS 255
Rudolph Harris	HFS-255
Linda Kahl	HFS-255
Antonia Mattia	HFS-255
Robert Martin	HFS-255
Charles Mize	HFS 255
Karin Ricker	HFS-255


Subject: Product Under Development/Glucosamine

This meeting was requested by Dr. Joseph Borzelleca on behalf of Cargill Acidulants to consult with FDA regarding use of glucosamine for use in beverages. This was the second meeting between FDA and Cargill. The visitors briefly discussed safety and efficacy studies of glucosamine, including Absorption, Distribution, Metabolism, and Excretion (ADME) studies, placebo use in clinical studies, Accepted Daily Intake (ADI) assessments, animal toxicity data, and other studies conducted in humans (which included human clinical studies for treatment of arthritis and glucosamine's effect on blood glucose levels). The company briefly addressed the National Toxicology Program (NTP) nomination for glucosamine, noting that the nomination was due to its widespread use and a lack of chronic toxicity data. The visitors concluded their presentation by summarizing the conclusion of their GRAS Expert Panel. FDA had no further comments regarding Cargill's presentation.

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DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Washington, DC 20204

June 12, 2007

FD



Edward A. Steele
EAS Consulting Group, LLC
1940 Duke Street, Suite 200
Alexandria, Virginia 22314

Re. GRAS Notice No. GRN 224

Dear Mr. Steele:

The Food and Drug Administration (FDA) has received the notice, dated May 15, 2007, that you submitted on behalf of ATLA Holdings, LLC (ATLA), 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 May 15, 2007, filed it on May 22, 2007, and designated it as GRN No. 000224

The subject of the notice is trans-resveratrol. The notice informs FDA of the view of ATLA that trans-resveratrol is GRAS, through scientific procedures, for use as an ingredient in bottled water products at levels up to 10 milligrams per liter.


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 homepage of the Office of Food Additive Safety (on the Internet at <http://www.cfsan.fda.gov/~lrd/foodadd.html>). If you have any questions about the notice, contact me at 301-436-1237.

Sincerely yours,

~~K. Ricker~~

Karin Ricker, Ph.D.
Division of Biotechnology and
GRAS Notice Review
Center for Food Safety
and Applied Nutrition

(b) (5)



**Memorandum of phone conversation**

Date: July 10, 2007

Time: 11:30 am- 12:00 noon

Between:

Dr. Mike DiNovi, HFS 255
Ms. Marilyn Diaz, HFS 255
Dr. Paulette Gaynor, HFS 255
Mr. Jeremy Mihalov, HFS 255
Dr. Robert Martin, HFS 255
Dr. Karin Ricker, HFS 255
Dr. Luis Valerio, HFS 255

And

Dr. Vivek Rajagopal, ATLA Holdings LLC
Mr. Greg Lamps, ATLA Holdings LLC
Mr. Edward Steel, EAS Consulting Group
Dr. Stanley Tarka, EAS Consulting Group
Dr. Madhu Soni, EAS Consulting Group

RE: GRN 224 trans- resveratrol


On May 15, 2007, FDA received a GRAS notification on *trans*-resveratrol for use in bottled water and designated it as GRN 224. On July 5, 2007, FDA contacted Mr. Ed Steele to notify him of FDA's concerns with the received notice and to set up a meeting time for further discussion.

In a conference call on July 10, 2007, FDA explained to the company that, based on material the company submitted, as well as additional scientific material FDA reviewed, FDA can not concur with the company's decision that *trans*-resveratrol is GRAS under the intended use in bottled water. FDA's conclusion was based primarily on the review of available toxicology data. FDA provided examples that lead to its conclusion, such as effects on the activity of cyclooxygenases *in vivo* and *in vitro*, and insufficiency of toxicology studies provided in the notice on potential for developmental and reproductive effects. FDA further concluded that at this time, there does not appear to be consensus in the scientific community about the safety of *trans*-resveratrol, and that the state of science relevant to its use as a food ingredient is still developing. In addition, FDA noted that the notice did not include a discussion of the source plant and possible toxic contaminants. FDA explained that the company can withdraw the notice or receive a letter with FDA's conclusion that the notice does not provide a sufficient basis for a GRAS determination

Karin Ricker

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**MEMORANDUM OF TELECONFERENCE CALL**

Date: November 2, 2006
Time: 10:00 AM to 11:00 AM EST
Place: 4300 River Rd., College Park, MD 20740, Rm. 2013
Teleconference Dial-In Number: 1- 888-452-9848

Participants:Industry:

Dr. Vivek Rajagopal	ATLA Holdings, LLC
Dr. Hitinder Gurm	ATLA Holdings, LLC
Dr. Madhu Soni	Kendle Consulting

FDA:

Robert Martin, Ph.D.	HFS-255
Ron Chanderbhan, Ph.D	HFS-255
Mike DiNovi, Ph.D.	HFS-255
Jeremy Mihalov, Ph.D.	HFS-255
Edmundo Garcia Jr.	HFS-255

Subject: Product under development.

This meeting was originally scheduled for September 20, 2006. Due to Dr. Barnett's (original consultant) passing, it was rescheduled for November 2, 2006.

Dr. Soni gave a brief overview of ATLA's product and went over the information that was submitted prior to our meeting. This information is attached.

Members of FDA's Office of Food Additive Safety (OFAS) advised that if ATLA did decide to submit a GRAS Notification, it should include information addressing the safety of the source of their product. The submission should also include a discussion of any other information that may appear to be inconsistent with the GRAS determination. OFAS added that generally, a GRAS Notification includes an adequate description of the proposed uses of the product including the identification of the food categories in which it will be used.

ATLA representatives thanked OFAS for their time and comments and stated that they would be contacting us if they had any further questions.

Edmundo Garcia Jr

Attachment

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MEMORANDUM OF MEETING

Date: December 15, 2006
Time: 1:30 - 2:30 p.m.
Place: FDA, Center for Food Safety and Applied Nutrition, Office of Food Additive Safety,
Room 2073, 4300 River Road, College Park, MD 20740
Subject: Product under development

Participants:

Visitors

Yoshihisa Katsuragi, Kao Corporation
Mistuhiko Katashima, Kao Corporation
Taisuke Itoh, Kao Corporation
Dave Muenz, Kao Corporation
Joseph Borzelleca, Professor Emeritus, Medical College of Virginia
Sandi Dennis, Morgan Lewis & Bockius, LLP
Sharon Segal, Morgan Lewis & Bockius, LLP
Paul Sheives, Morgan Lewis & Bockius, LLP

FDA

Negash Belay	HFS-255
Jeremy Mihalov	HFS-255
Ron Chanderbhan	HFS-255
Robert Martin	HFS-255
Luis Valerio	HFS-255

The meeting was requested by Morgan Lewis & Bockius, LLP, on behalf of Kao Corporation (Kao), to consult with FDA regarding GRAS status of a food ingredient (green tea catechin) under development. The visitors provided a presentation that included information about identity of the ingredient, method of manufacture, specifications, safety studies, intended use and intake estimates. The safety and identity characterization studies they discussed included Kao's own unpublished safety studies on Kao's


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ingredient and published safety and compositional studies on substances that Kao considers to be substantially equivalent to its ingredient. The visitors stated that Kao intends to use the results of its unpublished safety studies as corroborative data for the purpose of a GRAS determination, although the company plans to publish the studies.

With regard to identity, Dr. Valerio pointed out that the polyphenols component of the Kao ingredient is significantly lower than is found in the substances that Kao considers to be substantially equivalent to its ingredient. Dr. Martin stated that it is essential for Kao to address this issue in its GRAS determination. Dr. Valerio also pointed out that Kao needs to address the aspect of metabolic effects in its safety assessment. In addition, Dr. Valerio asked if Kao has obtained data on stability of its ingredient. The visitors indicated that Kao has such data

Negash Belay, Ph D

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DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
College Park, MD 20740

June 21, 2007

FD 

Stephen Paul Mahinka
Morgan, Lewis & Bockius LLP
1111 Pennsylvania Avenue, NW
Washington, DC
20004

Re: GRAS Notice No. GRN 000225

Dear Mr. Mahinka:

The Food and Drug Administration (FDA) has received the notice, dated May 17, 2007 that you submitted on behalf of Kao Corporation (Kao) 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 May 18, 2007, filed it on May 24, 2007, and designated it as GRN No. 000225.

The subject of the notice is catechins from green tea extract. The notice informs FDA of the view of Kao that catechins from green tea extract is GRAS, through scientific procedures, for use as an ingredient in beverages, including bottled teas, sport drinks, carbonated soft drinks and juice, at levels according to current good manufacturing practices.

In accordance with proposed 21 CFR 170.36(f), a copy of the information in the notice that conforms to the information described in proposed 21 CFR 170.36(c)(1) is available for public review and copying on the homepage of the Office of Food Additive Safety (on the Internet at <http://www.cfsan.fda.gov/~lrd/foodadd.html>). If you have any questions about the notice, contact me at (301)436-1198.

Sincerely yours,

Negash Belay, Ph.D.
Division of Biotechnology and
GRAS Notice Review
Center for Food Safety
and Applied Nutrition

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FILE
COPY

(b) (5)		(b) (6)		OFFICE	SURNAME	DATE	OFFICE	SURNAME	DATE



June 21, 2007

Stephen Paul Mahinka
Morgan, Lewis & Bockius LLP
1111 Pennsylvania Avenue, NW
Washington, DC
20004

Re: GRAS Notice No. GRN 000225

Dear Mr. Mahinka:

The Food and Drug Administration (FDA) has received the notice, dated May 17, 2007 that you submitted on behalf of Kao Corporation (Kao) 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 May 18, 2007, filed it on May 24, 2007, and designated it as GRN No. 000225.

The subject of the notice is catechins from green tea extract. The notice informs FDA of the view of Kao that catechins from green tea extract is GRAS, through scientific procedures, for use as an ingredient in beverages, including bottled teas, sport drinks, carbonated soft drinks and juice, at levels according to current good manufacturing practices.

In accordance with proposed 21 CFR 170.36(f), a copy of the information in the notice that conforms to the information described in proposed 21 CFR 170.36(c)(1) is available for public review and copying on the homepage of the Office of Food Additive Safety (on the Internet at <http://www.cfsan.fda.gov/~lrd/foodadd.html>). If you have any questions about the notice, contact me at (301)436-1198.


Sincerely yours,

Negash Belay, Ph.D.

Division of Biotechnology and
GRAS Notice Review
Center for Food Safety
and Applied Nutrition

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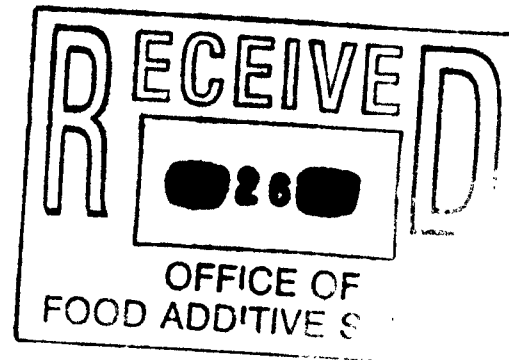


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Morgan, Lewis & Bockius LLP
1111 Pennsylvania Avenue, NW
Washington, DC 20004
Tel: 202.739.3000
Fax: 202.739.3001
www.morganlewis.com

Morgan Lewis
C O U N S E L O R S A T L A W

Stephen Paul Mahinka
Partner
202.739.5205
smahinka@morganlewis.com



November 26, 2007

BY HAND DELIVERY

Office of Food Additive Safety (HFS-255)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740

Re: Generally Recognized as Safe (GRAS) Notice for Green Tea Catechin, GRAS Notice No. 000225 - Kao Corporation

Dear Sir or Madam:

On behalf of our client, Kao Corporation (Kao), of Tokyo, Japan, we hereby withdraw our GRAS Notice for Green Tea Catechin, filed on May 17, 2007.

Subsequent to the filing of the GRAS Notice, Kao has completed additional clinical studies, which it believes would be useful additions to its GRAS Notice. In addition, Kao is contemplating modifications in the scope of contemplated food uses of its Green Tea Catechin product from those set out in its GRAS Notice.

Consequently, Kao requests that the Agency withdraw its GRAS Notice. Kao likely intends to refile a GRAS Notice for Green Tea Catechin in the future with appropriate additional studies and other modifications.

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Office of Food Additive Safety
November 26, 2007
Page 2

Please do not hesitate to contact me if you have any questions.

Sincerely,



Stephen Paul Mahinka

cc: Negash Belay, Ph.D. (via email)
Division of Biotechnology and GRAS Notice Review, HFS-255
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
Food and Drug Administration

000158

Belay, Negash

From: Belay, Negash
Sent: Friday, March 28, 2008 2:45 PM
To: 'smahinka@morganlewis.com'
Cc: Gaynor, Paulette M; Martin, Robert L; Chanderbhan, Ronald F; Danam, Rebecca *;
Srinivasan, Jannavi; Dinovi, Michael J
Subject: RE: Kao Corp. GRAS Notice - Summary of Suggestions and Cites

Dear Mr. Mahinka,

In a telephone conversation on November 19, 2007, we discussed with you the status of our review of GRAS Notice No. GRN 000225 GRAS (GRN 225) that was submitted by you on behalf of Kao Corporation (Kao). The subject of the notice is catechins from green tea extract. In that discussion, we informed you that, while our review of the notice was ongoing, we became aware of a report by the United States Pharmacopeia Dietary Supplements Information Expert Committee (USP) that raises safety concerns about the use of green tea catechins. The USP expressed its concern about potential adverse effects of green tea catechins on the liver and proposed a requirement for a cautionary statement on the label of dietary supplement products containing green tea extracts. In light of this development, we informed you of your options with regards to the status of GRN 225 and you indicated that Kao would withdraw the notice and address these safety concerns. We also informed you that we have identified various other insufficiencies in Kao's notice, to be communicated to you at a later time. The withdrawal of Kao's notice was subsequently confirmed by your letter dated November 26, 2007.

On February 29, 2008, you came in for a meeting with us to discuss the additional insufficiencies (i.e., aside from the USP issue) of the withdrawn notice and to inform us about your efforts to address the USP decision. At this meeting you indicated that Kao intends to address all outstanding issues and resubmit the notice. The purpose of this e-mail is, as agreed in our February 29 meeting, to convey to you a compilation of the various additional insufficiencies we identified from our review of GRN 225.

GENERAL COMMENTS:

1. No scientific name of the botanical source for the notified substance is provided.
2. Although the subject of the notice is a purified substance, much of the discussion in the notice is about green tea/green tea extract. The discussion needs to be centered on Kao's purified substance and its intended use in food.
3. Kao designates certain information on Page 5 and Pages 8-9 of the notice as confidential. The use of information claimed as undisclosable is inconsistent with GRAS determination criteria.
4. Kao provides publications in Japanese as part of the notice but has not included English translations of those publications.
5. Kao's information on use levels is not clear. A use level of 540 mg per beverage is indicated on page 18 of the notice while a self-limiting use level is also described on page 19.
6. With regard to additional reports (i.e., aside from the USP report) raising safety concerns, those identified below under Toxicology comments are being provided only as examples. Kao needs to address, in a comprehensive manner, all reports in the scientific literature that raise safety concern.

TOXICOLOGY COMMENTS:

The 28-day oral (gavage) toxicity studies of Green Tea Catechins (GTC) prepared for beverages in rats evaluated the potential adverse effects of 3 preparations of GTC,

1. Heat sterilized (GTC-H)

2. Unheated (GTC-UH)

3. Decaffeinated (GTC-HDC)

This study reveals the following:

1. At higher doses (1-2g/kg bw/day) of GTC-H, there is a significant decrease in body weight and food consumption, which interestingly, seems to be a distinct effect observed only in the male rats.
2. Similarly, the weights of Spleen, Testis (dose related) Pituitary and Thyroid/Parathyroid glands are significantly reduced in male rats. The authors considered these organ weight differences as spurious, incidental and unrelated to the administration of test articles.
3. In female rats, there is an increase in the weight of Thymus and longer activated thromboplastin time in the decaffeinated and unheated GTC groups respectively.
4. Minimal glandular stomach erosions were observed in both male and female rats (GTC-H).
5. Histological examination of the dead female rat receiving 2g/kg/d GTC-UH revealed extensive necrosis in one liver lobe and mild degenerative changes in the other.

FDA's observations on the 28-day oral (gavage) study:

The changes observed in male rats are consistent with other reports. Since it is a 28 day study and the number of animals in each group is only 5, the significant differences in organ weights will either become more significant or less meaningful in a longer term study with more animals (10-20) per study group. For example, the decrease in food consumption may be correlated to lack of appetite due to changes seen in the endocrine glands. Hence, these differences cannot be ignored.

The minimal glandular stomach erosions appear to be relevant and consistent in the context of gastrointestinal irritation observed in humans consuming high doses of tea preparations.

The authors did not discuss the hepatotoxic effects of GTC although they observe an increase in the Glutamyl transferase activity in female rats.

The following are the possible toxic effects reported by other studies that were not discussed:

1. Several case reports of hepatotoxicity related to the consumption of high doses of tea-based dietary supplements (10-29 mg/kg/d) Ann. Intern. Med. 144, 68-71, 2006.
2. Acute liver failure induced by green tea extracts: case report and review of the literature. Liver Transpl. 2006, 12:1892-5.
3. Cellular and in vivo hepatotoxicity caused by green tea phenolic acids and catechins. Free Rad. Biol. Med. 2006, 40:570-580.
4. A case of hepatotoxicity caused by green tea in a 51-year-old woman from Naples, Italy. Free Radic. Biol. Med. 43, 474, 2007.
5. In vitro hepatotoxicity: Hydro-alcoholic green tea extracts (80% ethanolic dry extracts) at a concentration of 1-3 mg/ml exerted acute cytotoxic effects in rat liver cells. Epigallocatechin-3-gallate (EGCG) was the major contributor to the cytotoxic effect suggesting the hepatotoxic potential of EGCG. The bioavailability and the exposure play a critical role in exerting the toxic effects. Food Chem. Toxicol. 43, 307-314, 2005.
6. Fasting increases the bioavailability of EGCG.
7. The uptake of (-)-Epigallocatechin-3-gallate, (EGCG), the most predominant catechin in GTC is highest and can induce toxicity in the liver, kidneys and intestine.
8. Toxicity in the liver appears to be more predominant in female rats and female dogs (Food Chem. Toxicol. 44, 636-650, 2005).
9. Possibility of individuals with a polymorphism in a key biotransformation pathway for

the tea polyphenols, such as low activity of COMT (catechin-O-methyltransferase) which increases exposure to the unmetabolized parent compound.

10. Involvement and interaction of potentially hepatotoxic pharmaceutical agents such as acetaminophen or other dietary supplements should be considered in humans.

11. Gastric carcinogenesis in rats: This study demonstrates that the combined administration of GTC (1%) and sodium nitrite (0.2%) selectively increased the incidence and multiplicity of neoplastic lesions in the forestomach of the rat after initiation with MNNG (N-methyl-N'-nitro-N-nitrosoguanidine). It also caused significant increases in 8-hydroxyguanosine levels in DNA indicative of oxidative damage. The average daily intakes of GTC were 432-580 mg/kg/day.

The overall data imply that excessive simultaneous intake of green tea and sodium nitrite (dietary nitrate from meats, vegetables and tap water by oral micro flora, food additives) might be a potential human risk, particularly in patients with reflux esophagitis. Cancer Sci 98, 949-957, 2007.

12. Goitrogenic effects: In a 13 week study, goiters were observed in F344 rats administered GTC in their diets. The incidence of thyroid lesions were higher in males than in females. The NOEL of GTC was considered to be 0.625% in males and 1.25% in females, based on histological changes of the thyroid. Arch Toxicol. 75, 591-596, 2001.

13. Teratogenicity and reproductive toxicity:

(a) Tea EGCG, classified as weakly embryotoxic, induces caudal regression in developing rat embryos even at much lower doses. $Ma150 = 54.2 \text{ mg/L}$; $IC50Ma1 = 45.8 \text{ mg/L}$. (Free Radical Biology & Medicine 43: 519-527, 2007) Doses as low as 25 mg/L triggered axial rotational defects and caudal regression and defects in brain and heart. The pro-oxidant effects of EGCG were evident and correlated with increased 8-isoprostane concentrations.

(b) Omitted in Kao's discussion in the notice is also the study by Isbrucker et al.; Safety studies on epigallocatechin gallate (EGCG) preparations. Part 3: teratogenicity and reproductive toxicity studies in rats. This in vivo study reported reduced growth rate in the offspring and slight decrease in the number of pups (even in the 2nd generation). The NOAEL was equivalent to 200 mg/kg/d (including lactating dams). Food Chem. Toxicol. 44:651-61, 2006.

(c) It is also reported that high tea consumption diminishes salivary 17 beta-estradiol concentration in Polish women. Br. J. Nutr. 95:989-995, 2006.

14. Fetal Leukemia Risk:

Dietary flavonoids induce cleavage in the MLL gene and may contribute to infant leukemia. PNAS 97, 4790-4795, 2000.

EGCG was the most abundant catechin in the placenta (3077.4 pmol/g) and the fetus (159.3 pmol/g). Although these levels are much lower than those required to induce chromosomal translocation, further studies are needed in vivo to establish the increased risk, if any, of leukemia due to maternal flavonoid consumption.

Toxicology's overall conclusion:

In light of the above findings, further studies (for example a 90-day study with 10-20 animals/group, peer reviewed & published) or additional scientific information would provide a better understanding of the potential adverse effects of GTC/polyphenols.

CHEMISTRY COMMENTS:

1. Structural Formula of Catechin monomers:

(a) The structure is unclear (is it a C or an O?) - Page 6.

2. Manufacturing Process:

A. Method of Manufacture:

(a) Product description: It varies from Type 1/Type 2, to heated/unheated, to GTC/Tannase-treated GTC to GTC/UT GTC. There needs to be consistency through out the document. The submission also reverses the 'Types' in the discussion (Page 8-9, Page 14-15).

(b) Unclear if Figure 1 is description of Type 1 process? Also, unclear if Active Carbon in Figure 1 is an 'adsorbing' agent or 'absorbing agent'? - Page 8.

(c) Section III(V) has been referred to in the text along with reference Footnote 18. They do not seem to correlate. Sec III(V) discusses levels of catechin monomers in various tea manufacturing processes while Footnote 18 discusses a 28-day toxicity study with the untreated GTC along with product characteristics for tannase treated GTC- out of place! - Page 9.

(d) Figure 2, Page 9, has speculations such as 'probable' and 'accidental'. Requires rewriting or a method-based LOD specification.

(e) Purity of tannase is 0.9%? - Page 9.

(f) Footnote 17 refers to Section III (E) for 'difference in manufacturing processes'. Notifier describes the differences in product characteristics in that section - Page 9.

3. Specifications:

(a) The specification for total catechin monomer is very wide considering the method is HPLC based. Notifier could provide method description - Page 12.

(b) Appendix H: In the three lots' information provided, GTC and Tannase-GTC form 50% and 70%, with 'other components' making 20-30%. What is in the remaining product?

(c) Notifier has set the microbiological and heavy metal specifications for the beverage form. Is the product tested after manufacture and if so what are the manufacturing testing specifications prior to formulation in beverage? - Page 12.

4. Analytical methods:

(a) What are the HPLC assays and their specificity in the total catechin monomer method?

(b) How do the JFSL methods compare with standard methods?

(c) Tartaric acid method description is unclear in its purpose and action.

5. Product Characteristics:

(a) What is the HPLC method to analyze catechin monomers?

(b) It is unclear as to what the 'derived Na2C calorimetric' method is?

(c) The tannase treated GTC seems to have same w/w% of catechins with gallate moiety as the untreated GTC. But the text states that the function of the tannase is to remove these moieties? (Page 9).

(d) Are 'other polyphenols' quantifiable from HPLC? Also, Scale in the figure is not visible.

6. Stability in beverages:

(a) GTC in Beverage: Conclusion should be 6 months at 25 deg C and 2 months at 37 deg C not 'for at least 6 months at temperatures up to 37 deg C' - Page 18. (Also, 540 mg total catechin in the product is mentioned here)

(b) Unclear about GTC Type tested in Oolong tea beverage - Page 18

7. Self Limiting Levels

(a) Define 'high levels'.

8. Consumption levels and EDI calculation:

(a) Using information through out the document (stability data and footnotes) we gathered the use levels to be 540mg of catechins in 500 ml (max beverage size discussed in the text). Is this correct?

We hope this information is of help to you and contact us again if you have further questions.

Negash

Negash Belay, Ph.D.
Division of Biotechnology and GRAS Notice Review, HFS-255
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
Food and Drug Administration

-----Original Message-----

From: smahinka@morganlewis.com [mailto:smahinka@morganlewis.com]
Sent: Friday, March 07, 2008 9:49 AM
To: Belay, Negash
Cc: Kathleen M. Sanzo; Sharon Segal; Dr. Joseph F. Borzelleca
Subject: Kao Corp. GRAS Notice - Summary of Suggestions and Cites

Negash:

Thank you again for your efforts in arranging what all Kao Corporation representatives believe was a very valuable meeting.

Following our meeting, Kao has confirmed that it definitely intends to refile a GRAS Notice. It intends to refile by the end of March since, as we discussed timing is critical with respect to this product.

Consequently, although we recognize it is a great imposition on your schedule, we would greatly appreciate receiving as soon as possible the brief summary list of the comments and suggestions and of the cites to suggested articles important to include., so that Kao can promptly prepare and submit a new GRAS Notice and Dr. Borzelleca and his panel can prepare a revised Expert Panel Report.

We have, for example, reviewed recent articles for which Lambert is an author, but is difficult for us to determine which ought to be the focus of our consideration as identified by FDA in its prior review.

We greatly appreciate your time in providing this brief summary as soon as possible, so that we might promptly provide a comprehensive and acceptable revised Notice.

Best regards.

Steve

Sent from my BlackBerry Handheld. (Morgan Lewis)

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you have received this communication in error, please
notify us immediately by e-mail and delete the original
message.

MEMORANDUM OF MEETING

Date: January 31, 2008

Time: 10:00 a.m. – 11:00 a.m.

Place: FDA, Center for Food Safety and Applied Nutrition, Office of Food Additive Safety, 4300 River Road, College Park, MD 20740

Subject: Product under development

Participants:Visitors:

Joseph F. Borzelleca	Medical College of Virginia
Melody Harwood	Cantox Health Sciences, International
Noriko Iwaki	Pharma Foods International Co., Ltd.
Yusuke Sauchi	Pharma Foods International Co., Ltd.
T.J. Tang	Pharma Foods International Co., Ltd.
Yoshikazu Yoshidu	Mitsubishi Corporation
Yoshiaki Yoshikuni	Pharma Foods International Co., Ltd.

FDA:

Negash Belay	HFS-255
Ronald Chanderbhan	HFS-255
Michael DiNovi	HFS-255
Paulette Gaynor	HFS-255
Robert Martin	HFS-255
Moraima Ramos Valle	HFS-255
Jannavi Srinivasan	HFS-255
Timothy Twaroski	HFS-255

Cantox Health Sciences International requested the meeting on behalf of Pharma Foods International Company, to consult with FDA regarding the ingredient *gamma*-Amino butyric acid (GABA).

The visitors described the basis for their GRAS determination. They discussed the method of manufacture, chemical identity, and specifications for GABA. They also discussed the proposed uses, exposure estimates, and safety information.

In response to the visitor's description of the chemical purity of GABA, FDA representatives noted that the FAO/WHO Joint Expert Committee on Food Additives recommends a purity of 95% for a single chemical entity, and if less than 95%, all components should be characterized.


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In response to the visitor's description of the exposure estimates, FDA representatives suggested that it would be helpful to include calculations of the Japanese intake levels.


During the discussion of safety studies, the visitors noted that the Environmental Protection Agency (EPA) had issued an exemption for tolerance for GABA for use as a pesticide. In support of that exemption, EPA referenced animal toxicity studies. The visitors stated that they would obtain the literature cited by EPA by making a Freedom of Information Act request. The visitors stated that this literature was not reviewed by the company's expert panel.

In addition to the comments above, FDA representatives provided further comments on issues that would need to be addressed in a GRAS Notification for GABA. FDA representatives noted that there are other safety-related issues for GABA including hypotensive effects, potential for electrolyte imbalance, potential use in treatment of schizophrenia, and the popularity of GABA as a dietary supplement among body-builders looking to increase their endogenous levels of human-growth hormone. FDA representatives also suggested that it would be useful to know more about the microbial production system especially with respect to methods used to assure the exclusion of the microbe from the final product. Finally, FDA representatives suggested that it would be helpful to address biogenic amines and ethyl carbamate and why the visitors believe that these compounds would not present a safety problem for the GABA product.

At the close of the meeting, FDA representatives observed that some of the information presented appears to be clinical studies relating to GABA. Because of this observation, FDA representatives mentioned the recent amendments (i.e., the Food and Drug Administration Amendments Act of 2007; FDAAA) to the Federal, Food, Drug, and Cosmetic Act, in particular section 912 of the FDAAA.


Susan J. Carlson, Ph.D.

(b) (5)



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DEPARTMENT OF HEALTH & HUMAN SERVICES



Public Health Service

Food and Drug Administration
College Park, MD 20740

September 9, 2008

Yoshiaki Yoshikuni, Ph.D.
Pharma Foods International Co., Ltd.
1-49 Goryo-Ohara, Nishikyo-Ku
Kyoto, 615-8245
JAPAN

Re: GRAS Notice No. GRN 000257

Dear Dr. Yoshikuni;

The Food and Drug Administration (FDA) has received the notice, dated July 22, 2008, that you 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)). FDA received this notice on August 7, 2008, filed it on August 11, 2008, and designated it as GRN No. 000257.

The subject of the notice is gamma-amino butyric acid (GABA). The notice informs FDA of the view of Pharma Foods International Co., Ltd. that gamma-amino butyric acid (GABA) is GRAS, through scientific procedures, for use as an ingredient in beverages and beverage bases, chewing gum, ready-to-drink coffee and tea products, and candy at levels ranging from 0.04% to 4%.


In accordance with proposed 21 CFR 170.36(f), a copy of the information in this notice that conforms to the information described in the proposed GRAS exemption claim (proposed 21 CFR 170.36(c)(1)) is available for public review and copying on the homepage of the Office of Food Additive Safety (on the Internet at <http://www.cfsan.fda.gov/~lrd/foodadd.html>). If you have any questions about the notice, contact me at 301-436-1253.

Sincerely yours,

Susan J. Carlson, Ph.D.
Division of Biotechnology and
GRAS Notice Review
Center for Food Safety
and Applied Nutrition

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MEMORANDUM OF TELECONFERENCE

Date: November 13, 2008

Time: 2:00 p.m. – 3:00 p.m.

Place: FDA, Center for Food Safety and Applied Nutrition, Office of Food Additive Safety, 4300 River Road, College Park, MD 20740

Subject: GRN 000257, *gamma*-Amino butyric acid (GABA)

Participants:Telephone:

Melody Harwood	Cantox Health Sciences, International
Ryan Simon	Cantox Health Sciences, International
Karen Young	Cantox Health Sciences, International

FDA:

Negash Belay	HFS-255
Susan Carlson	HFS-255
Ronald Chanderbhan	HFS-255
Michael DiNovi	HFS-255
Paulette Gaynor	HFS-255
Aydin Orstan	HFS-255
Moraima Ramos-Valle	HFS-255
Timothy Twaroski	HFS-255

After brief introductions, FDA personnel opened the meeting by stating that the purpose of the meeting was to discuss numerous issues with the notice as submitted that would preclude the agency from issuing a “no questions” letter. A detailed discussion of the issues uncovered by the review team then followed.

The FDA microbiology reviewer discussed issues that he found during his review. The first issue was from the notice’s statement of intended uses. The reviewer stated that the notice excluded the use of *gamma*-Amino butyric acid (GABA) from meat. The reviewer suggested that if the notifier wished to exclude products regulated by the U.S. Department of Agriculture, the exclusion should use the terms “meat and poultry products.”

The second issue noted by the FDA microbiology reviewer had to do with the method of manufacture. According to the notice, the method of manufacture included an ultra-filtration step using a 0.5 micron filter. The reviewer stated that this would not ensure the exclusion of live microorganisms as stated in the notice. The reviewer noted that the method of manufacture did include a 97°C heat treatment for 30 minutes that would exclude all live microorganisms. The reviewer also noted that the units describing the

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ultra-filtration were incorrect. The correct unit is micron (designated as “μ”) not micromolar designated as “μM.”

The third and final issue discussed by the FDA microbiology reviewer concerned the detection of biogenic amines during the manufacturing process. The reviewer noted that the method of manufacture described in the notice included steps for measuring ethyl carbamate. The reviewer stated that ethyl carbamate forms from citrulline in the presence of alcohol, therefore it would be more appropriate to measure citrulline during the manufacturing process. Alternatively, the notice could specifically exclude alcoholic beverages from the list of intended uses.

The discussion continued with chemistry issues. The FDA chemistry reviewer noted that Table 5 within the notice detailed exposure estimates on a per body weight basis. According to the reviewer, when he worked through the calculations he got some non-standard values for body weight, including some values that seemed to be rather high for particular groups. The reviewer suggested that these values be more thoroughly explained and that it would be helpful if the codes used in the calculations were included in the notice. Ms. Harwood replied that the values in Table 5 were calculated by averaging the exposure estimates for individuals in the National Health and Nutrition Examination Survey (NHANES).

The FDA chemistry reviewer also discussed the lack of a cumulative exposure estimate. He suggested that inclusion of this estimate would be helpful. The FDA toxicology reviewer added that the lack of a cumulative dietary exposure estimate was problematic. As described in the notice, the background dietary exposure (not including the new intended uses) to GABA is quite high. The publicly available toxicological studies cited within the notice are for dietary levels of GABA below the cumulative exposure (background as well as proposed new use levels). There are additional studies cited within the notice at higher GABA levels, closer to the cumulative dietary exposure estimate; however those studies are not publicly available. The notifier cites its unpublished studies and additional studies cited by the U.S. Environmental Protection Agency (EPA) in support of one of its decisions. Since none of these studies is publicly available, the reviewer noted that this leads to a problem supporting the general recognition of safety.

Ms. Harwood replied that Cantox had tried to locate the published study cited by EPA; however they had had no success and that the notifier's GRAS panel did not think it was critical. Ultimately, they believed that the metabolic data were critical to establishing the general recognition of safety while the toxicological studies were not and furthermore that the metabolic data were well-established in the public literature.

The FDA toxicology reviewer concurred that the metabolic data were compelling. The FDA toxicologist and chemist suggested that the notice needed to include a more extensive narrative, outlining why certain lines of evidence were more supportive of the general recognition of safety than others. The FDA toxicology reviewer elaborated that the Estimated Daily Intake and Acceptable Daily Intake values are the reverse of what

would be expected to be seen when dietary exposure estimates are part of the general recognition of safety and that the reasons why this may not be relevant to establishing safety are not adequately discussed.

The FDA chemistry reviewer noted that he could not reproduce the calculations in Table 3. Ms. Harwood acknowledged that the values in Table 3 were not clearly presented. Finally, the reviewer inquired as to the source of the iron particles that had to be cleared from the preparation using magnets, as outlined in the method of manufacture. Ms. Harwood stated that they would have to ask their client. The FDA microbiology reviewer remarked that the presence of iron could imply that there was some sort of contamination occurring during manufacturing.

The FDA toxicology reviewer returned to a discussion of the toxicological issues. He stated that the notice did a good job addressing the use of GABA in dietary supplements. The reviewer advised focusing the toxicological safety discussion on what is known about the physiology and metabolism of GABA (specifically Absorption, Distribution, Metabolism, Excretion studies known as “ADME”) while relying on the dietary exposure estimates as secondary data supportive of safety. He added that it would be helpful if they could find the study cited by EPA.

Ms. Harwood inquired as to how EPA’s opinion was viewed by FDA in terms of establishing the general recognition of safety. FDA personnel clarified that in order to establish the consensus opinion of experts, all of the information must be publicly available. In other words, if a panel of experts reviews data that is not publicly available and subsequently renders an opinion regarding safety, even if the experts are well-recognized, the opinion does not meet the general recognition of safety for GRAS ingredients because the data were not publicly available.

The FDA toxicology reviewer reiterated that the main problem with the notice is that the cumulative estimated dietary exposure values are above the values used in the publicly available toxicological studies. He also suggested that perhaps the dietary exposure estimates for GABA are too high and need to be re-evaluated. The FDA toxicologist added that the narrative of the notice needs to be reworked so as to adequately address the differences in the dietary exposure values in the published versus the unpublished literature.

Ms. Harwood returned to the discussion of calculating the dietary intakes. She requested that the FDA chemist call Dr. Karen Young of Cantox directly to discuss FDA’s views on these calculations.

FDA personnel mentioned that the notice contained specifications for GABA that were in Japanese and that there were also citations for Japanese articles. FDA personnel explained that FDA had no assurances that the GRAS panel had reviewed English translations of these documents. Ms. Harwood explained that the GRAS panel had not requested them. Mr. Simon asked if FDA needed certified translations to which FDA

personnel replied yes. FDA personnel also suggested that Cantox make a Freedom of Information Act request for the studies cited by EPA.

At the close of the meeting, FDA review scientists summarized different options for submitting an improved notice and meeting the general recognition of safety:


- Publish the unpublished toxicological studies cited in the notice.
- Reevaluate the background exposure estimates.
- Find the published studies cited by EPA.
- Establish the safety of GABA by emphasizing the ADME data.

As a follow-up to this teleconference, FDA and Cantox corresponded by electronic mail concerning the exposure estimates (attachment).


Susan Carlson, Ph.D.

Attachment: Electronic mail correspondence string between Cantox and FDA

(b) (5)



000160

Carlson, Susan

From: Dinovi, Michael J
Sent: Wednesday, December 10, 2008 2:37 PM
To: Carlson, Susan
Subject: FW: exposure assessments

My reply, Susan

Mike

From: Dinovi, Michael J
Sent: Tuesday, December 09, 2008 12:32 PM
To: 'Karen Young'
Subject: RE: exposure assessments

Hi Karen

Sorry to miss you yet again on Friday, and I was out sick yesterday.

In general, you would include a naturally occurring background intake if you are adding a "food use" to the total. It should be relatively straightforward in the discussion: If the added is dwarfed by the natural, no problem, if the added is on par or dwarfs the natural, the safety of the added intake has to be supported by the available data. Some nod must be made to the background intake, even if only a crude estimate is available, as it would let you classify the data need.

On the body weight thing, I hadn't seen the tables before the call, so I was taking a quick look. I thought it was the teen males that came out at about 90. If it is all adult males, it is less likely to be mistaken

Hope this is helpful

Mike

From: Karen Young [mailto:kyoung@cantox.com]
Sent: Thursday, December 04, 2008 1:24 PM
To: Dinovi, Michael J
Cc: Melody Harwood; Larry McGirr; kmusaveloso@cantox.com; Ryan Simon
Subject: RE: exposure assessments

Hi Mike,

It is good to finally connect with you!

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Thank you for your email. Sorry if I was not clear. What I meant in terms of background sources was food sources of the compound that are *naturally occurring*. So, do we need to address total dietary exposure from naturally occurring background sources + proposed uses? This is the issue that was raised during our call (I believe that by then you had to be at another meeting and were no longer on the call). For GABA, there are currently no other food uses however, there are naturally occurring sources, which were presented. Supplement use was not one of the proposed uses for GABA.

As we discussed on the call, we use the actual body weights of each individual in the NHANES survey when we calculate intakes on a per kg body weight basis. I took a look at the summary tables that were presented and did a backward calculation also. When I did this, female teens were below 60 kg while male teens were just above. Perhaps you were referring to male adults who ranged from ~80 to 90 kg? Total population average was just over 60 kg also. Nonetheless, it would not be a problem to include the actual food code list that we use for our assessment.

Thanks again for your help.

Karen

From: Dinovi, Michael J [mailto:michael.dinovi@fda.hhs.gov]
Sent: Thursday, December 04, 2008 12:50 PM
To: Karen Young
Cc: Melody Harwood; Larry McGirr; kmusaveloso@cantox.com
Subject: RE: exposure assessments

Hi Karen (and everyone else I have now added as CC:s)

Yes, the phone tag has been entertaining!

I don't suppose Ashley mentioned my reply to his related question the other day? We cannot require anything, as this is a voluntary program and we don't want to frighten anyone away. Having said that, we would typically tell any notifier that their submission would have to address the total dietary exposure from new and current uses. How else could you conclude that the uses were safe, without a notion of what total exposure is. My recollection of the call was that there was a question of adding supplement use (or was it medical food use) to the proposed uses. That's a different matter, as you won't have access to food consumption data to do that. I assume that we are all using variations on the same software theme to get these exposures (we use FARE, which we have licensed from Exponent). It would be trivial to add old food uses to proposed uses to do an exposure. I questioned the relationship between the numbers in the tables for exposure on a kg-bw basis and on a g/d basis. The ratio of the means must arithmetically be the mean body weight of the surveyed population and as I recall, it suggested that teens would average about 90 Kg, which isn't possible. That was why we asked you to send us the code set that you used, so we could crosscheck and see what if any problem there was. You have to remember (in light of the voluntary thing above) that we really only act as the advocate for the public and must be as skeptical as necessary whenever we look at data.

I'm not sure that this gets you anywhere, but please do feel free to keep trying me, or failing that, give me a specific time to call you and we can all chat. We don't want this program to be adversarial, as I am sure you (and CanTox) know, so any help I can give is a pleasure. Tomorrow is a good day for me (the rest of today is pretty busy).

Take care and talk to you soon

Mike

From: Karen Young [mailto:kyoung@cantox.com]

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Sent: Thursday, December 04, 2008 12:34 PM
To: Dinovi, Michael J
Cc: Larry McGirr; Melody Harwood; Kathy Musa-Veloso
Subject: exposure assessments

Hello Mike,

We've been playing phone tag for a bit, so I thought it would be best to send you an email.

The issue of how to most appropriately conduct our intake assessments came up during our call regarding the GRAS status for GABA after you had left the room. Another scientist on the call had stated that we should present estimates for cumulative exposure from both background sources and proposed food uses. Traditionally we have not done this; we have presented estimates for intakes from proposed food uses (using NHANES data) and discussed data on background dietary consumption from published sources. Do you now require future submissions to provide estimates for cumulative exposure from both background and proposed food uses? If so, would a crude, but conservative, estimate suffice (*i.e.*, adding estimates from published sources to estimates that were calculated using NHANES data)? Or, would an assessment using NHANES data to estimate cumulative exposure from both background and proposed uses be required?

Thank you for your help in clarifying this issue.

Best regards,
Karen

Karen W. H. Young, Ph.D.
Scientific and Regulatory Consultant
Food and Nutrition
CANTOX HEALTH SCIENCES INTERNATIONAL

2233 Argentia Road, Suite 308
Mississauga, ON L5N 2X7
CANADA
Tel: 905-542-2900, extension 308
Fax: 905-542-1011
kyoung@cantox.com

www.cantox.com

000163



Carlson, Susan

From: Carlson, Susan
Sent: Wednesday, January 14, 2009 3:30 PM
To: 'y-yoshikuni@pharmafoods.co.jp'
Subject: GRAS Notice 000257 Withdrawal letter
Attachments: withdrawal letter.pdf

Dear Dr. Yoshikuni,

Attached to this e-mail is an Adobe Acrobat file of the Food and Drug Administration's acknowledgement of your withdrawal of GRN 000257. The original copy of the letter is being sent to you via the U.S. Postal Service.

Sincerely,
Susan Carlson, Ph.D.

U.S. Food and Drug Administration
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
Division of Biotechnology and GRAS Notice Review

000170

Memorandum of Meeting

PR



Date: September 10, 2007
Time: 10:00 - 11:00 a.m.
Place: University Station, 4300 River Road, Room 2013
Subject: Pre-submission meeting for lupin derived ingredients

Participants:Industry:

Joseph F. Borzelleca	Medical College of Virginia
Fiona Fleming	George Weston Foods
Melody Harwood	Cantox

FDA:

Ronald Chanderbhan	HFS-255
Michael DiNovi	HFS-255
Marcella Fruchter	HFS-255
Paulette Gaynor	HFS-255
Jeremy Mihalov	HFS-255

Ms. Melody Harwood requested the meeting to discuss submitting a notice in accordance with the Food and Drug Administration's proposed regulation, proposed 21 CFR 170.36 (62 FR 18938; April 17, 1997; Substances Generally Recognized as Safe (GRAS). The visitors presented an overview of the company (George Weston Foods/Weston Technologies) and of the data and information that the company is relying on to support its view that the intended use of the six lupin derived ingredients (various types of flour, proteins, or fibers) are GRAS.

FDA representatives inquired whether the visitors knew what alkaloids would be present in the ingredients, reminding them to include that information in the submission. We also asked whether the ingredient would be used in meat and poultry products because such uses would result in an USDA review, suggesting the visitors check with USDA for any specifics that they need for their review. FDA representatives suggested separating the six ingredients into more than one submission.

The visitors stated that lupin-derived ingredients may cross react with peanuts. The visitors also stated that a recent EFSA opinion amended the list of known allergens to include lupin, suggesting that the United States could do the same, identifying the source as is done with soy, for example. FDA representatives stated that because of the cross-reactivity to peanut, people will be concerned about these ingredients. In response to the visitors question as to how protein cross-reactivity is handled, we offered to provide a contact for the FALCPA group. Subsequent to the meeting, we provided contact information for Felicia Billingslea.

Paulette Gaynor, Ph.D.

(b) (5)

FILE
COPY

OFFICE	SURNAME	DATE	OFFICE	SURNAME	DATE	OFFICE	SURNAME	DATE
(b) (5)								

PRE-NOTIFICATION MEETING FOR LUPIN-DERIVED INGREDIENTS

Monday, September 10, 2007 at 10 a.m.

**4300 River Rd., Room 2073
College Park, MD 20740**

Participants: CFSAN Representatives
Ms. Fiona Fleming, George Weston Foods
Dr. Joseph F. Borzelleca, Medical College of Virginia
Ms. Melody Harwood, Cantox Health Sciences International

- 1. Introductions**
- 2. Overview of George Weston Foods / Weston Technologies**
- 3. Basis for GRAS**
 - (a) Manufacturing, Chemistry, Specifications**
 - (b) Uses and Exposure**
 - (c) Data Supporting GRAS status**
- 4. Next Steps**

Gaynor, Paulette M

From: Gaynor, Paulette M
Sent: Monday, July 16, 2007 3:35 PM
To: Fruchter, Marcella I
Subject: FW: Request for Pre-Notification Meeting - Lupin-derived ingredients
Attachments: Draft FDA Meeting Agenda.doc

Marcella,

Please serve as CSO for this meeting.

Thank you,

Paulette

From: Martin, Robert L
Sent: Monday, July 16, 2007 7:26 AM
To: 'Melody Harwood'; Gaynor, Paulette M
Subject: FW: Request for Pre-Notification Meeting - Lupin-derived ingredients

Ms. Harwood, by way of this e-mail message, I am forwarding your request for a pre-submission meeting to Dr. Paulette Gaynor who will assign it to someone in her group to contact you and arrange this meeting. Someone from her group will be contacting you soon.

Thanks.
Robert L. Martin
301-436-1219

From: Melody Harwood [mailto:mharwood@cantox.com]
Sent: Monday, July 16, 2007 1:13 AM
To: Martin, Robert L
Subject: Request for Pre-Notification Meeting - Lupin-derived ingredients

Dear Dr. Martin,

I am contacting you on behalf of our client, Weston Technologies, a division of George Weston Foods Limited, who would like to schedule a meeting with representatives of the Administration to go over the self-affirmation of the Generally Recognized As Safe (GRAS) status of their lupin-derived food ingredients prior to submitting a GRAS Notification. Below, please find information that might be useful for scheduling the meeting.

- Name of Company: Weston Technologies, a division of George Weston Foods Limited, Enfield, Australia
- Attendees: Ms. Fiona Fleming (Weston Technologies), Dr. Steve Taylor (University of Nebraska), and myself, Melody Harwood (CANTOX)

- The products are derived from sweet varieties of *Lupinus* spp. (lupin) (*L. angustifolia*, *L. albus*, *L. luteus*, and *L. mutabilis*) and include lupin flour, two lupin protein fractions, two lupin kernel fibres, and lupin hull fibre.
- The lupin-derived food ingredients will be marketed for use in various traditional food products such as bakery products, breakfast cereals, and beverages.
- The objectives of the meeting are to provide an overview of the company and the basis for GRAS of the lupin-derived ingredients under the intended conditions of use.
- Equipment needs: In-focus or other projection machine to connect to a laptop for visual presentation

A draft agenda is attached for your review. I would like to propose a meeting on September 10 or 11, 2007, as our client is in North America from Australia for a short period of time during September and unfortunately, these dates are the only ones that seems to fit her schedule. I do hope that it also is suitable for yourself and representatives of your department. If not, please let me know what alternate dates would be convenient, and perhaps I can re-arrange my schedule to accommodate.

<<Draft FDA Meeting Agenda.doc>>

I would like to thank you in advance for your assistance in this matter. If you require further information, please do not hesitate to contact me by telephone or via email. I look forward to hearing from you at your earliest convenience.

Kindest regards,

Melody

Melody Harwood, M.Sc.
Associate Director, Business Development
Food and Nutrition
CANTOX HEALTH SCIENCES INTERNATIONAL

2233 Argentia Road, Suite 308
Mississauga, ON L5N 2X7 CANADA
Tel: 905-542-2900, extension 302
Fax: 905-542-1011
Mobile: 905-580-6693
mharwood@cantox.com

PRE-NOTIFICATION MEETING FOR LUPIN-DERIVED INGREDIENTS

DRAFT AGENDA

September 10 or 11, 2007

**4300 River Rd.
College Park, MD 20740**

Participants: CFSAN Representatives - To be Determined
Ms. Fiona Fleming, Weston Technologies
Dr. Steve Taylor, University of Nebraska
Ms. Melody Harwood, CANTOX Health Sciences International

- 1. Introductions**
- 2. Overview of Weston Technologies/George Weston Foods**
- 3. Basis for GRAS**
 - (a) Manufacturing, Chemistry, Specifications**
 - (b) Uses and Exposure**
 - (c) Data Supporting GRAS status**
- 4. Next Steps**

MEMORANDUM OF TELECONFERENCE

Date: December 4, 2008

Time: 2:00 p.m. – 2:45 p.m.

Place: FDA, Center for Food Safety and Applied Nutrition, Office of Food Additive Safety, 4300 River Road, College Park, MD 20740

Subject: GRN 000262, sweet lupin protein; GRN 000263 sweet lupin fiber; GRN 000264, sweet lupin flour

Participants:Telephone:

Sherry Duckworth	George Weston Foods, Ltd.
Cathy Fryirs	George Weston Foods, Ltd.
Fiona Fleming	George Weston Foods, Ltd.

FDA:

Susan Carlson	HFS-255
Ronald Chanderbhan	HFS-255
Michael DiNovi	HFS-255
Bianca Farias	HFS-255 (ORISE Fellow)
Paulette Gaynor	HFS-255
Molly Harry	HFS-255 (ORISE Fellow)
Stefano Luccioli	HFS-200
Sylvester Mosley	HFS-255
Vladimir Yurovsky	HFS-255

Following introductions, FDA personnel opened the meeting by thanking the representatives of George Weston Foods, Ltd. for their submissions to FDA's Generally Recognized as Safe (GRAS) Notification Program. FDA personnel stated that one of the purposes of the GRAS Notification Program is to establish a dialogue between FDA and industry and that FDA appreciated the efforts of George Weston Foods, Ltd. staff in putting together their GRAS submissions.

FDA personnel then proceeded to explain why the notices failed to meet the standards for general recognition of safety. During the review process, FDA staff noted that it is well-established in the scientific literature that lupin proteins are similar enough to peanut proteins that peanut-allergic individuals may also react to lupin-containing products. Because of this cross-reactivity, peanut allergic individuals could potentially have life-threatening reactions when they eat lupin-containing products for the very first time. As there is little consumption of lupin-containing products in the United States, the peanut-allergic population is unaware of this danger. FDA staff stated that ingredient labels

000148

containing only the term, “sweet lupin” as proposed in the notices, would not be adequate to warn U.S. consumers of the risk of a reaction if they are peanut-allergic.

FDA personnel then covered the administrative options available under the GRAS Notification program for notices that fail to establish that an ingredient is generally recognized as safe for its intended uses. FDA personnel explained that any notifier may ask FDA to cease evaluation of a notice. In response to these requests, FDA posts an acknowledgement of the receipt of the request on FDA’s Internet site alongside the GRAS Notice number. In these cases, FDA’s acknowledgement uses standard language with no mention of the reasons why the notifier is requesting that FDA cease to evaluate the notice. Alternatively, FDA may issue a letter stating that FDA does not agree with the notifier’s conclusion that an ingredient is GRAS for its intended uses and post this letter on the FDA Internet site.

In discussing these administrative options, FDA personnel noted that the notices had been filed and assigned as GRN 000262, sweet lupin protein; GRN 000263, sweet lupin fiber; and GRN 000264, sweet lupin flour. FDA personnel went on to explain that George Weston Foods, Ltd. would not be receiving the standard acknowledgement letter that FDA sends out upon the filing of GRAS notices as FDA staff had difficulty determining appropriate names for the subject of GRN 000262. For its GRAS notice correspondence, FDA uses a technically descriptive name for the subject of the notice. In the case of GRN 000262, FDA reviewers were unable to determine enough of the details of the manufacturing process and subsequent physical/chemical characteristics of the subject to be able to assign a descriptive name.

FDA reviewers then briefly highlighted the technical issues that they noted during their reviews. Due to the detailed nature of these issues, FDA agreed to send a list of issues by electronic mail at a later date (electronic mail correspondence of February 23, 2009).

In concluding the discussion of the administrative details, FDA personnel stated that in their review of the lupin literature they had noticed that lupin flour may be yellow and used to mimic the addition of butter or eggs to baked goods. FDA personnel stated that under U.S. law, any new ingredient that is added to food with the intention of imparting color may be an unapproved color additive and may not be GRAS. The appropriate regulatory process in these instances is the color additive petition process.

Following the discussion with the review scientists, FDA personnel reiterated the main concern with the notices was the fact that a certain percentage of individuals who are allergic to peanuts will also be allergic to lupin. Given the lack of lupin consumption in the U.S. population, there is no awareness of this cross-reactivity among peanut allergic individuals and this presents a significant safety issue.

At the close of the meeting, FDA personnel and George Weston Foods, Ltd. representatives discussed administrative items, including the process for withdrawing a GRAS notice and the process for resubmitting a GRAS notice.

~~~~
Susan Carlson, Ph.D.

(b) (5) 

000150



Carlson, Susan

From: Sherry.Duckworth@gwf.com.au
Sent: Monday, February 23, 2009 3:26 PM
To: Carlson, Susan
Subject: RE: Lupin GRAS Notices - reviewer's comments

Attachments: lupin comments.doc



lupin comments.doc
(62 KB)

Dear Susan

Thank you for collating the comments from the reviewers. Our team will review them and let you know if we have any questions. We would certainly meet with you prior to submitting a new GRAS Notice.

Regards
Sherry

"Carlson, Susan"
<Susan.Carlson@fd
a.hhs.gov>

24/02/2009 06:46
AM

<Sherry.Duckworth@gwf.com.au>

To

cc

Subject
RE: Lupin GRAS Notices - reviewer's
comments

Dear Sherry,

Here are the collated comments from our reviewers that were raised in our teleconference with you on December 4, 2008. As we stated at the meeting, given the complexities of these notices, we would strongly encourage you to meet with us prior to making any new GRAS Notice submissions for lupin.

My apologies for the delay. Thank you for your patience. Please don't hesitate to contact us if you have any questions.

Regards,
Susan

-----Original Message-----

From: Sherry.Duckworth@gwf.com.au [mailto:Sherry.Duckworth@gwf.com.au]
Sent: Friday, February 20, 2009 6:37 AM
To: Carlson, Susan

000157

Subject: RE: Lupin GRAS Notices - reviewer's comments

Dear Susan

Have you had any success in obtaining the comments from the supervisor?

Thanks
Sherry

"Carlson, Susan"

<Susan.Carlson@fd

a.hhs.gov>

To

<Sherry.Duckworth@gwf.com.au>

24/01/2009 08:32

cc

AM

Subject

reviewer's

RE: Lupin GRAS Notices -
comments

Hello Sherry,
Thank you for inquiring. The comments are with one of the supervisors. I
will gently remind him.
Thank you for your continued patience.
--Susan

-----Original Message-----

From: Sherry.Duckworth@gwf.com.au [mailto:Sherry.Duckworth@gwf.com.au]
Sent: Friday, January 23, 2009 4:31 PM
To: Carlson, Susan
Subject: Lupin GRAS Notices - reviewer's comments

Dear Susan

Just wondering how you are progressing with compiling the reviewer's
comments.

Thanks

Sherry

Sherry Duckworth
Project Manager
Research and Technology
George Weston Technologies
(A Division of George Weston Foods)
1 Braidwood Street
Enfield NSW 2136
Australia
Tel: + 61 2 9764 8160
Fax: + 61 2 9742 5959
MOB: +61 0419 412 398
Email: sherry_duckworth@gwf.com.au
----- Forwarded by Sherry Duckworth/WT/NSW/GWF on 24/01/2009 08:11 AM

Sherry

Duckworth/WT/NSW/

GWF

To

"Carlson, Susan"

21/12/2008 06:17

<Susan.Carlson@fda.hhs.gov>

PM

cc

Subject

reviewer's

Re: Lupin GRAS Notices -

comments(Document link: Sherry

Duckworth)

Thanks Susan. We will look forward to receiving them.

"Carlson, Susan"

<Susan.Carlson@fd

a.hhs.gov>

To

<Sherry.Duckworth@gwf.com.au>

20/12/2008 09:48

CC

AM

Subject

Lupin GRAS Notices - reviewer's
comments

Dear Sherry,

We are still trying to get our reviewers' comments together for you. The reviewers are working on them (and they are around for at least Monday and Tuesday of this coming week). We hope to get them to you soon. Thank you for your continued patience.

Regards,
Susan

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(See attached file: lupin comments.doc)

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000161

KEY:

GRN 000262 Lupin Protein

GRN 000263 Lupin Fiber

GRN 000264 Lupin Flour

All review team members discussed the issue of cross-reactivity between lupin protein allergens and allergens in peanuts. Review team members noted that lupin protein is present in the flour and fiber ingredients (the subjects of GRN 000264 and GRN 000263) in addition to the protein ingredient (GRN 000262). The review team believes that this cross-reactivity is a safety issue for peanut allergic individuals in the U.S. who have no experience with lupin products and would have no way of knowing that they could already be sensitized to lupin and thus at risk.

The chemistry reviewers listed the following concerns from the notices:

- For GRN 000262, the identity section does not cover the following—
 - Protein name, molecular weight, sequence information.
 - The active component of the product (globulin or albumin?).
- For GRN 262 the following details are not clearly stated in the method of manufacture—
 - The chemical identity of Fractions 1 and 2, i.e. name of the protein in these fractions (alpha, beta, gamma conglutin). (Gamma conglutin has only been mentioned in the acute tox section (Page 15)).
 - The adjusted pH value or range (alkaline or acidic).
- Identities of the sweet varieties of the four species of sweet lupin that are used as source materials are unclear. What quality control procedures are used in the method of manufacture?
- The manufacturing processes described for GRN 000262 and GRN 000264 would not be expected to remove glycoalkaloids, mycotoxins, or saponins. Is there a preliminary soaking or boiling step?
- Maximum alkaloid and phomopsin levels in lupin flour (<200mg/kg and 5 mcg/kg, respectively), as set forth in GRN 000264, are the same as those of the Advisory Committee on Novel Foods and Processes (ACNFP) of the United Kingdom, (UK) and in the Australia New Zealand Food Standards Code. These levels appear to have been set based on limited information about exposure and toxicity of these natural toxicants. The notifier should address the following in a discussion of the general recognition of safety of intended uses of lupin ingredients:
 - Estimated cumulative intakes of sweet lupin from the three ingredients (flour, fiber, protein) are 92.6 g/p/d at the mean and 158.5 g/p/d at the 90th percentile. These intakes, assuming a 200 mg/kg glycoalkaloid level in sweet lupin and 70 kg human, are associated with glycoalkaloid exposures as high as 265 mcg/kg/d and 453 mcg/kg/d at the 90th percentile. These alkaloid exposures would be much greater than the tolerable exposure level of 35 mcg/kg/d tentatively established by ANZFA (2001) (cited on p. 000043 of GRN 000264.) A similar calculation can be performed for phomopsins, although no tolerable level has been set.

- The proposed food ingredient uses of lupin (as flour, fiber, protein) are more extensive than those currently reported in Europe and considered in the FSANZ review. For example, in GRN 000264, proposed use level (up to 25% replacement of other sources of flour)--exceeds that considered by Australia New Zealand Food Authority (ANZFA) (up to 10%); proposed level may be above realistic use level.
- For GRN 000262, the specifications section does not address the following –
 - microbiological specifications for consistency lots.
- For GRN 000264, batch analyses show yeast and mold specification not consistently met (p. 000113).
- For GRN 000264, one of the references (Bradbury et al., 2004) is confidential.
- For GRN 000264, we have noticed that due to the presence of carotenoids, lupin flour may be used to mimic the addition of eggs. Under U.S. law, this use would likely be regulated as a color additive, requiring the submission of a color additive petition.
- The detection method used to determine the level of phomopsins needs to be discussed. Also, the level of exposure is not well-explained.
- In the discussion of pesticide residue analysis, the notifier mentions several MRL standards for various grains. Further, this is not a comprehensive list of pesticides with MRLs for grains or for lupin. The notifier should clearly explain the justification for choosing these comparison standards and specific pesticides for analysis. Further, the Limit of Detection (LOD) values for certain pesticides are above the Maximum Residue Levels (MRL) set by FSANZ in 2005. The notifier cannot state that pesticides are present at or below the MRL. In a GRAS submission, we would expect reference to valid analytical methods with appropriate LODs.
- GRN 000262 does not include a discussion of the stability and metabolic fate of lupin protein. Such a discussion might include–
 - Protein digestibility with reference to the EFSA document that discusses possible resistance to digestion.
 - The heat stability of the protein.
- In GRN 000262, issues regarding Estimated Intake of Sweet Lupin Fractions 1 and 2 that should be addressed include–
 - Use of Fraction 1 in meat.
 - The use of lupin protein in infant formula (Page 13, Para 3).
 - Background protein consumption is 75.2 g (mean). In the expert panel opinion (Pg 3), the discussion of a conservative estimate of all uses + all users is 92.6 g. This implies that it is greater than the background (worst case scenario). Please clarify apparent inconsistency.
- In GRN 000262's 'Other Phytonutrient' section (Pg 23), there is a comparison made to soy in infant formula. If sweet lupin protein is intended to be used in infant formula, this should be clearly stated.

The toxicology reviewers then summarized the highlights of their concerns:

- For sweet lupin flour (GRN 000264), the test substance used in most of the safety studies cited in the notice were whole lupin seeds or lupin protein. There were three studies summarized in the notice on flour from individual lupin species. There was no study in the notice that used lupin flour derived from a mix of lupin species and varieties which is the subject of GRN 000264. In order for the studies to support the safe use of sweet lupin flour, the test article of the studies must be similar to the subject of the GRAS notice.
- The subchronic rat study (using *L. angustifolia* flour) had no concurrent controls and the data were compared with historical values from another laboratory. There were two human studies using *L. albus* flour. One study gave one (150 g) cookie/day enriched with lupin flour to subjects for 60 days and found no significant adverse effect. The second study, in men only, reported an average nitrogen digestibility level of 77% and an increase in plasma urea nitrogen.
- For GRN 000264, lupin is reported to be low in lysine, methionine, cysteine and calcium. George Weston Foods, Ltd. needs to state clearly the levels of these amino acids and calcium in the flour.
- For GRN 000264, *Lupinus albus* seed is reported to be very high in manganese and may be toxic. This was not addressed in the notice.
- For GRN 000262 and GRN 000263 phomopsin levels are not presented in the batch analyses presented in Appendix B-2. Moreover, the notifier did not provide the correct reference to the maximal established level of 5 ppb. The FSANZ document that the notifier refers to (http://www.foodstandards.gov.au/_srcfiles/TR1.pdf), states that in the absence of human data and in the absence of a NOEL in animals, it is not possible to derive a tolerable level for human exposure.
- Also for GRN 000262 and GRN 000263, more sensitive methods should be used to determine the levels of pesticide residues so that the established MRL can be met. Their levels may be <0.05 ppm as detected, but >0.02 ppm as established by FSANZ (Table B-3-1).
- For GRN 000263, subchronic toxicity data on lupin flour cannot support the safety of lupin hull fiber.
- For GRN 000262, the reviewer noted some minor issues. In Table 4 footnote b is missing. On P. 24 line 2, maybe 1.4 mg trypsin inhibitors/day? In Appendix B-4, Table B-3-1, why does the amount of insoluble fiber decrease during storage?

MEMORANDUM OF MEETING

Date: August 26, 2009

Time: 2:00 p.m. – 2:30 p.m.

Place: FDA, Center for Food Safety and Applied Nutrition, Office of Food Additive Safety, 4300 River Road, College Park, MD 20740, room 2073

Subject: GRN 000262, sweet lupin protein; GRN 000263 sweet lupin fiber; GRN 000264, sweet lupin flour

Participants:Visitor:

Melody Harwood

Cantox Health Sciences International

FDA:

Susan Carlson

HFS-255

Robert Martin

HFS-255

The meeting was held at the request of Ms. Harwood on behalf of Cantox's client, George Weston Foods, Ltd., for the purpose of discussing the steps needed to resubmit the previously withdrawn GRAS Notices for sweet lupin protein, sweet lupin fiber, and sweet lupin flour (Attachment). Ms. Harwood stated that George Weston Foods, Ltd. wished to pursue labeling as a condition of safe use for their lupin-based ingredients.

FDA personnel noted that because it has been established in the scientific literature that peanut-allergic individuals also react to lupin-based ingredients we believe it will be difficult to establish a basis for the general recognition of safety for lupin, even with a label statement.

FDA personnel and Ms. Harwood discussed the possibility of submitting food additive petitions instead of GRAS Notices. FDA personnel cautioned Ms. Harwood that given the well-known cross-reactivity of lupin proteins and peanut proteins and the lack of consumer awareness in the U.S. market, it would be difficult to establish the safe use of lupin ingredients, even with a regulation.

Ms. Harwood thanked FDA personnel for their time and stated that she would convey the information to George Weston Foods, Ltd.


Susan Carlson, Ph.D.

Attachment

(b) (5)



000165

Attachment

From: Melody Harwood
To: Carlson, Susan;
cc: Martin, Robert L;
Subject: RE: Request for Meeting on Lupin October 30, 2009
Date: Monday, August 24, 2009 3:45:45 PM

OK, sounds great. Thanks for your consideration. I look forward to seeing you on Wednesday.

Kind regards,
Melody

-----Original Message-----

From: Carlson, Susan [<mailto:Susan.Carlson@fda.hhs.gov>]
Sent: Monday, August 24, 2009 1:48 PM
To: Melody Harwood
Cc: Martin, Robert L
Subject: RE: Request for Meeting on Lupin October 30, 2009

Dear Melody,
Please plan on spending a few minutes (5 to 10 min) with Dr. Martin and myself following your meeting here Wednesday, August 26. We would like to discuss our current thinking on the allergenicity issues of lupin.
Thank you,
Susan

-----Original Message-----

From: Melody Harwood [<mailto:mharwood@cantox.com>]
Sent: Monday, August 24, 2009 12:01 PM
To: Carlson, Susan
Cc: Ashley Roberts
Subject: Request for Meeting on Lupin October 30, 2009

Hi Dr. Carlson,

I hope this finds you well and enjoying summer time in Maryland.

I am contacting you to request a meeting with our client, George Weston Foods, Australia, to discuss some of the issues that were identified by the agency during their review last fall of 3 GRAS Exemption Claims for their lupin ingredients. In particular, the current issue that our client is facing pertains to potential allergenicity of the lupin

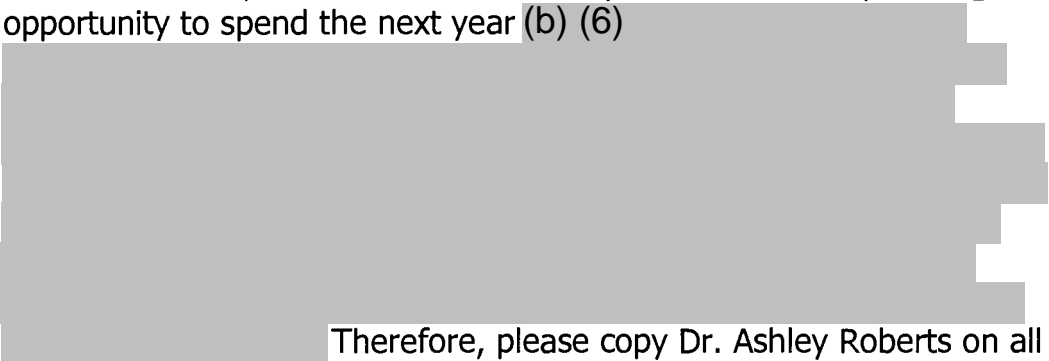
000166

proteins, as well as potential cross-reactivity of these proteins with other allergens (including peanut) and how to adequately label retail products containing these ingredients so as to properly inform any lupin- or peanut-sensitive individuals.

Our client has been working with several experts, including Dr. Steve Taylor, various clinicians, and members of Cantox, and would like to request a meeting with CFSAN to discuss this matter with all that need to be involved. It is requested that representatives from both OFAS and ONLDS be invited to attend this meeting, and I want to get your opinion if we should also invite a representative of the Office of Regulations, Policy and Social Sciences or of the Office of Compliance, as I believe this is an unprecedented issue? Perhaps even Michael Landa, Deputy Director for Regulatory Affairs, should be included? Our client will be coming from Australia and have already confirmed the availability of their other experts for October 30, 2009. I do realize that you usually need a couple of suggested days in order to find a date that suits the schedules of all involved, but I'm hoping that providing ample time before the meeting will allow for this date to be feasible to meet.

I look forward to your thoughts on this matter. I will be down in College Park on Wednesday, August 26th, and if there is an opportunity to discuss a meeting strategy with you and your colleagues I would be grateful and will gladly make myself available after 2 pm.

On another note, I am excited to inform you that I will be pursuing an opportunity to spend the next year (b) (6)



Therefore, please copy Dr. Ashley Roberts on all correspondence related to this meeting request, as he will continue to work with yourself and our client subsequent to my last day at Cantox to finalize a meeting date..

Please don't hesitate to let me know if you have any questions or concerns.

Kind regards,
Melody

Melody Harwood, M.Sc.
Associate Director, Business Development
Food and Nutrition
Cantox Health Sciences International

2233 Argentia Road, Suite 308
Mississauga, ON, L5N 2X7, CANADA
Tel: 905-542-2900, Extension 302
Fax: 905-542-1011
Mobile: 905-580-6693
mharwood@cantox.com

The 4th Practical Short Course on Functional Oils: Omega-3 Fatty Acids
Market Trends, Nutrition & Health, Utilization in Food Systems is August
24-25, 2009 in Chicago, IL! Ms. Lina Paulionis of Cantox will be
presenting, "Omega-3 Oils: Health Claims Global Perspectives" on August
24 at 10:00 a.m. Don't miss it!
<<http://home.scarlet.be/~tpm12374/smartshortcourses/pdf/4thFLipids.pdf>>
P Please consider the environment before printing this e-mail.

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Carlson, Susan

SU

**From:** Kane, Rhonda R.**Sent:** Thursday, October 08, 2009 9:45 AM**To:** Billingslea, Felicia B; June, Geraldine A; Martin, Robert L; Carlson, Susan; Dinovi, Michael J; Luccioli, Stefano; Tarantino, Laura M**Subject:** RE: Outstanding Industry Issues About Lupine

To all,

[REDACTED] ([REDACTED]
[REDACTED] b [REDACTED]
[REDACTED]) [REDACTED]
[REDACTED] ([REDACTED]
[REDACTED] 5 [REDACTED]
[REDACTED]) [REDACTED]

[REDACTED]

Rhonda R. Kane, MS, RD

Consumer Safety Officer (HFS-820)

Food Labeling and Standards Staff

ONLDS / CFSAN / FDA

CP 1, Room 4D-008

Phone: (301) 436-1803

Fax: (301) 436-2636

E-mail: Rhonda.Kane@fda.hhs.gov**From:** Carlson, Susan**Sent:** Monday, October 05, 2009 3:09 PM**To:** Kane, Rhonda R.**Cc:** Billingslea, Felicia B; June, Geraldine A; Martin, Robert L**Subject:** RE: Outstanding Industry Issues About Lupin

Dear Rhonda,

(b) (5) [REDACTED]
[REDACTED]

[REDACTED]

Thank you Rhonda,
--Susan**From:** Martin, Robert L**Sent:** Tuesday, September 29, 2009 7:42 AM**To:** Carlson, Susan**Cc:** Billingslea, Felicia B; June, Geraldine A; Kane, Rhonda R.**Subject:** RE: Outstanding Industry Issues About Lupin

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(b) (5)

Thanks.

Robert L. Martin

301-436-1219

From: Carlson, Susan

Sent: Monday, September 28, 2009 3:15 PM

To: Martin, Robert L

Cc: Billingslea, Felicia B; June, Geraldine A; Kane, Rhonda R.

Subject: FW: Outstanding Industry Issues About Lupin

Hello Bob,

(b) (5)

Thank you,
Susan

From: Kane, Rhonda R.

Sent: Monday, September 28, 2009 2:26 PM

To: Carlson, Susan

Subject: FW: Outstanding Industry Issues About Lupin

FYI

From: Dinovi, Michael J

Sent: Monday, September 28, 2009 12:54 PM

To: Kane, Rhonda R.

Subject: RE: Outstanding Industry Issues About Lupin

Rhonda

000170



(b) (5)

Mike

From: Kane, Rhonda R.
Sent: Monday, September 28, 2009 12:31 PM
To: Dinovi, Michael J
Subject: Outstanding Industry Issues About Lupin

Hi, Michael,

(b) (5)



Rhonda R. Kane, MS, RD
Consumer Safety Officer (HFS-820)
Food Labeling and Standards Staff
ONLDS / CFSAN / FDA
CP 1, Room 4D-008
Phone: (301) 436-1803
Fax: (301) 436-2636
E-mail: Rhonda.Kane@fda.hhs.gov

From: Billingslea, Felicia B
Sent: Monday, September 28, 2009 10:44 AM
To: 'Ashley Roberts'; Tarantino, Laura M
Cc: Ian Munro
Subject: RE: Meeting on Lupin

Hi Ashley,

I am aware of your requests and will ask one of my staff to follow up with you.

Thanks,
Felicia

From: Ashley Roberts [<mailto:aroberts@cantox.com>]
Sent: Wednesday, September 23, 2009 8:11 AM
To: Tarantino, Laura M
Cc: Ian Munro; Billingslea, Felicia B
Subject: RE: Meeting on Lupin

Dear Laura,

Many Thanks for your quick response and advice.

I will make contact with Felicia directly on this matter

Kind Regards

Ashley

000171

From: Tarantino, Laura M [mailto:Laura.Tarantino@fda.hhs.gov]
Sent: Wednesday, September 23, 2009 8:03 AM
To: Ashley Roberts
Cc: Ian Munro; Billingslea, Felicia B
Subject: RE: Meeting on Lupin

Hi, Ashley,

We had suggested that your initial contact be Felicia Billingslea in our labeling group, since the specific questions are primarily about labeling precedents. She or one of her staff will help arrange the meeting, and will keep Dr. Carlson and others in OFAS in the loop.

Laura Tarantino

From: Ashley Roberts [mailto:aroberts@cantox.com]
Sent: Tuesday, September 22, 2009 4:28 PM
To: Tarantino, Laura M
Cc: Ian Munro
Subject: FW: Meeting on Lupin

Dear Dr. Tarantino,

I just wanted to follow-up on some previous correspondence between Dr. Munro and yourself regarding the setting up of a meeting between the Agency, the manufacturer of lupin and Dr. Steve Taylor. Unfortunately, Dr. Munro has been out of the office recently and he has asked me to make contact with you again regarding this matter.

Dr. Munro has informed me that you recommended that we should try to set up a meeting by speaking firstly with one of your staff members whose name we are now seeking. I am led to believe that this person was not Dr. Carlson as outlined below. Please could you relay to me who this person was so that I might speak with them directly on this matter.

I look forward to receiving your response on this matter

Kind Regards

Ashley Roberts, PhD
VP, Food & Nutrition Group

CANTOX Health Sciences International
2233 Argentia Road, Suite 308
Mississauga, Ontario, Canada
L5N 2X7

T: 905-542-2900
F: 905-542-1011
E: aroberts@cantox.com
W: www.cantox.com

From: Ian Munro
Sent: Tuesday, July 14, 2009 2:17 PM
To: Tarantino, Laura M
Subject: RE: Meeting on Lupin

Hi Laura....Thanks for your email. No I am nor in Aspen this week but stuck working in Toronto. I have a hectic week this week but I would like to call you next week after Monday. So why don't I shoot you an email early next week and you can tell me a good time to call.....Thanks.....Ian

Ian C. Munro, Ph.D., F.A.T.S., FRCPath
Executive Vice President

000172

Senior Scientific Consultant
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imunro@cantox.com
www.cantox.com



Please consider the environment before printing this e-mail

From: Tarantino, Laura M [<mailto:Laura.Tarantino@fda.hhs.gov>]
Sent: Monday, July 13, 2009 4:10 PM
To: Ian Munro
Subject: RE: Meeting on Lupin

Hi, Ian,

Thank you so much for this background. It is very helpful. And I am sorry for my delay in responding, but I was on leave until last week, and I am just now catching up on email. Before I left, I did talk to a few folks, so I think we can work on setting up a meeting, and your notion of contacting Susan Carlson to coordinate is a good one. However, I'd be happy to discuss with you beforehand, if it will be helpful.

I am assuming you are in Aspen this week. I am not in College Park today, but should be there the rest of this week and most of next. I will plan to try to contact you early next week, or give me a holler before then if convenient.

Laura

From: Ian Munro [<mailto:imunro@cantox.com>]
Sent: Monday, June 29, 2009 10:26 AM
To: Tarantino, Laura M
Cc: Melody Harwood
Subject: RE: Meeting on Lupin

Laura,

Many thanks for your help so far. Here is a brief synopsis of what has happened to date.

We had a pre-submission meeting on September 10, 2007 with several OFAS people.

- Dr. Michael DiNovi, OFAS/DBGNR
- Dr. Ron Chanderbhan, OFAS/DBGNR
- Dr. Paulette Gaynor, OFAS/DBGNR
- Dr. Marcella Fruchter, OFDCER/DEC/COCB
- Dr. Jeremy Mihalov, OFAS/DBGNR

There didn't seem to be any concerns about the overall safety of lupin ingredients including lupin protein, flour and fibre until the potential allergenicity of lupin protein was mentioned, and even more so when the potential cross-reactivity with peanut was disclosed. Coming out of that meeting, it was recommended that we speak with a representative of the Office of Nutrition, Labelling and Dietary Supplements (ONLDS), so one of our people, Melody Harwood called and spoke with Rhonda Kane and explained the situation to her. As there currently isn't a policy for labelling for 'new' or cross-reactive allergens, she was not able to give clear guidance on what to do other than to comply with the ingredient labelling requirements, as per the regulations.

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We submitted the Notices for all three ingredients and yes, there were questions raised during the initial review phase (prior to withdrawal as a consequence of the allergen question), but none that were considered to be insurmountable. Our client indicated that the general feel they got in subsequent

discussions with OFAS was that the concerns of potential allergenicity and cross-reactivity were paramount and that the issue would not go away with simple conformity to the current ingredient and allergen labelling requirements, as these would not provide an avenue for warning or decreasing risk in lupin- or peanut-allergic individuals.

I believe that Dr. Susan Carlson handled the client's initial submission, and so we have planned to contact her to set up the meeting once we have settle on who should attend from your side

Thanks for your help.

Ian

From: Tarantino, Laura M [mailto:Laura.Tarantino@fda.hhs.gov]

Sent: Tuesday, June 16, 2009 3:22 PM

To: Ian Munro

Subject: RE: Meeting with CFSAN

Hello, Ian,

I did hear your voicemail. I have been, and am still today, away from my own office and travelling among buildings. I will give you a call back this week. I appreciate your wanting to address the cross-reactivity concern and willingness to work out something on labelling. Of course we will be happy to meet. We will have some discussion about who to have from our end. In the meantime, do let's reconnect later this week.

Best regards,

Laura

From: Ian Munro [mailto:imunro@cantox.com]

Sent: Tuesday, June 16, 2009 12:34 PM

To: Tarantino, Laura M

Subject: FW: Meeting with CFSAN

Dear Laura,

As per my voice mail of yesterday, I wanted to speak with you/get your feedback on the logistics of a meeting to discuss the labelling and self-affirmed GRAS status of our client's various lupin-derived ingredients.

These ingredients have been sa-GRAS, and the current issue that our client is facing pertains to potential allergenicity of the lupin proteins, as well as potential cross-reactivity of these proteins with peanut and how to adequately label retail products containing these ingredients so as to properly inform any lupin- or peanut-sensitive individuals. We have in the past discussed this concern with representatives of both the Office of Food Additive Safety and of the Office of Nutrition, Labeling and Dietary Supplements of CFSAN; however, this appears to be a situation without precedent under CFSAN policy and so a solution has not yet been reached.

Our client has been working with several experts, including Dr. Steve Taylor and various clinicians, as well as members of my staff, and would like to request a meeting with CFSAN to discuss this matter with all involved. It is anticipated that representatives from both OFAS and ONLDS would be invited to attend this meeting, and I want to get your opinion if we should also invite a representative of the Office of Regulations, Policy and Social Sciences or of the Office of Compliance. Perhaps even Michael Landa, Deputy Director for Regulatory Affairs, should be included?

I look forward to hearing from you at your earliest convenience. Many thanks in advance for your assistance with this matter.

Kind regards,

Ian

Ian C. Munro, Ph.D., F.A.T.S., FRCPATH
Executive Vice President
Senior Scientific Consultant
Cantox Health Sciences International
2233 Argentia Road, Suite 308
Mississauga, ON L5N 2X7
Phone: (905) 542-2900
Fax: (905) 542-1011
imunro@cantox.com
www.cantox.com



Please consider the environment before printing this e-mail

Are you attending NutrEvent in Lille, France, June 17-18, 2009?

Cantox's **Nigel Baldwin** will be speaking & participating in the Roundtable Discussion during the Regulatory Frameworks Seminar, June 17.

Memorandum of Meeting

PR



Date: September 10, 2007
Time: 10:00 - 11:00 a.m.
Place: University Station, 4300 River Road, Room 2013
Subject: Pre-submission meeting for lupin derived ingredients

Participants:Industry:

Joseph F. Borzelleca	Medical College of Virginia
Fiona Fleming	George Weston Foods
Melody Harwood	Cantox

FDA:

Ronald Chanderbhan	HFS-255
Michael DiNovi	HFS-255
Marcella Fruchter	HFS-255
Paulette Gaynor	HFS-255
Jeremy Mihalov	HFS-255

Ms. Melody Harwood requested the meeting to discuss submitting a notice in accordance with the Food and Drug Administration's proposed regulation, proposed 21 CFR 170.36 (62 FR 18938; April 17, 1997; Substances Generally Recognized as Safe (GRAS). The visitors presented an overview of the company (George Weston Foods/Weston Technologies) and of the data and information that the company is relying on to support its view that the intended use of the six lupin derived ingredients (various types of flour, proteins, or fibers) are GRAS.

FDA representatives inquired whether the visitors knew what alkaloids would be present in the ingredients, reminding them to include that information in the submission. We also asked whether the ingredient would be used in meat and poultry products because such uses would result in an USDA review, suggesting the visitors check with USDA for any specifics that they need for their review. FDA representatives suggested separating the six ingredients into more than one submission.

The visitors stated that lupin-derived ingredients may cross react with peanuts. The visitors also stated that a recent EFSA opinion amended the list of known allergens to include lupin, suggesting that the United States could do the same, identifying the source as is done with soy, for example. FDA representatives stated that because of the cross-reactivity to peanut, people will be concerned about these ingredients. In response to the visitors question as to how protein cross-reactivity is handled, we offered to provide a contact for the FALCPA group. Subsequent to the meeting, we provided contact information for Felicia Billingslea.

Paulette Gaynor, Ph.D.

(b) (5)

FILE
COPY

OFFICE	SURNAME	DATE	OFFICE	SURNAME	DATE	OFFICE	SURNAME	DATE
(b) (5)								

PRE-NOTIFICATION MEETING FOR LUPIN-DERIVED INGREDIENTS

Monday, September 10, 2007 at 10 a.m.

**4300 River Rd., Room 2073
College Park, MD 20740**

Participants: CFSAN Representatives
Ms. Fiona Fleming, George Weston Foods
Dr. Joseph F. Borzelleca, Medical College of Virginia
Ms. Melody Harwood, Cantox Health Sciences International

- 1. Introductions**
- 2. Overview of George Weston Foods / Weston Technologies**
- 3. Basis for GRAS**
 - (a) Manufacturing, Chemistry, Specifications**
 - (b) Uses and Exposure**
 - (c) Data Supporting GRAS status**
- 4. Next Steps**

Gaynor, Paulette M

From: Gaynor, Paulette M
Sent: Monday, July 16, 2007 3:35 PM
To: Fruchter, Marcella I
Subject: FW: Request for Pre-Notification Meeting - Lupin-derived ingredients
Attachments: Draft FDA Meeting Agenda.doc

Marcella,

Please serve as CSO for this meeting.

Thank you,

Paulette

From: Martin, Robert L
Sent: Monday, July 16, 2007 7:26 AM
To: 'Melody Harwood'; Gaynor, Paulette M
Subject: FW: Request for Pre-Notification Meeting - Lupin-derived ingredients

Ms. Harwood, by way of this e-mail message, I am forwarding your request for a pre-submission meeting to Dr. Paulette Gaynor who will assign it to someone in her group to contact you and arrange this meeting. Someone from her group will be contacting you soon.

Thanks.
Robert L. Martin
301-436-1219

From: Melody Harwood [mailto:mharwood@cantox.com]
Sent: Monday, July 16, 2007 1:13 AM
To: Martin, Robert L
Subject: Request for Pre-Notification Meeting - Lupin-derived ingredients

Dear Dr. Martin,

I am contacting you on behalf of our client, Weston Technologies, a division of George Weston Foods Limited, who would like to schedule a meeting with representatives of the Administration to go over the self-affirmation of the Generally Recognized As Safe (GRAS) status of their lupin-derived food ingredients prior to submitting a GRAS Notification. Below, please find information that might be useful for scheduling the meeting.

- Name of Company: Weston Technologies, a division of George Weston Foods Limited, Enfield, Australia
- Attendees: Ms. Fiona Fleming (Weston Technologies), Dr. Steve Taylor (University of Nebraska), and myself, Melody Harwood (CANTOX)

- The products are derived from sweet varieties of *Lupinus* spp. (lupin) (*L. angustifolia*, *L. albus*, *L. luteus*, and *L. mutabilis*) and include lupin flour, two lupin protein fractions, two lupin kernel fibres, and lupin hull fibre.
- The lupin-derived food ingredients will be marketed for use in various traditional food products such as bakery products, breakfast cereals, and beverages.
- The objectives of the meeting are to provide an overview of the company and the basis for GRAS of the lupin-derived ingredients under the intended conditions of use.
- Equipment needs: In-focus or other projection machine to connect to a laptop for visual presentation

A draft agenda is attached for your review. I would like to propose a meeting on September 10 or 11, 2007, as our client is in North America from Australia for a short period of time during September and unfortunately, these dates are the only ones that seems to fit her schedule. I do hope that it also is suitable for yourself and representatives of your department. If not, please let me know what alternate dates would be convenient, and perhaps I can re-arrange my schedule to accommodate.

<<Draft FDA Meeting Agenda.doc>>

I would like to thank you in advance for your assistance in this matter. If you require further information, please do not hesitate to contact my by telephone or via email. I look forward to hearing from you at your earliest convenience.

Kindest regards,

Melody

Melody Harwood, M.Sc.
Associate Director, Business Development
Food and Nutrition
CANTOX HEALTH SCIENCES INTERNATIONAL

2233 Argentia Road, Suite 308
Mississauga, ON L5N 2X7 CANADA
Tel: 905-542-2900, extension 302
Fax: 905-542-1011
Mobile: 905-580-6693
mharwood@cantox.com

PRE-NOTIFICATION MEETING FOR LUPIN-DERIVED INGREDIENTS

DRAFT AGENDA

September 10 or 11, 2007

**4300 River Rd.
College Park, MD 20740**

Participants: CFSAN Representatives - To be Determined
Ms. Fiona Fleming, Weston Technologies
Dr. Steve Taylor, University of Nebraska
Ms. Melody Harwood, CANTOX Health Sciences International

- 1. Introductions**
- 2. Overview of Weston Technologies/George Weston Foods**
- 3. Basis for GRAS**
 - (a) Manufacturing, Chemistry, Specifications**
 - (b) Uses and Exposure**
 - (c) Data Supporting GRAS status**
- 4. Next Steps**

MEMORANDUM OF TELECONFERENCE

Date: December 4, 2008

Time: 2:00 p.m. – 2:45 p.m.

Place: FDA, Center for Food Safety and Applied Nutrition, Office of Food Additive Safety, 4300 River Road, College Park, MD 20740

Subject: GRN 000262, sweet lupin protein; GRN 000263 sweet lupin fiber; GRN 000264, sweet lupin flour

Participants:Telephone:

Sherry Duckworth	George Weston Foods, Ltd.
Cathy Fryirs	George Weston Foods, Ltd.
Fiona Fleming	George Weston Foods, Ltd.

FDA:

Susan Carlson	HFS-255
Ronald Chanderbhan	HFS-255
Michael DiNovi	HFS-255
Bianca Farias	HFS-255 (ORISE Fellow)
Paulette Gaynor	HFS-255
Molly Harry	HFS-255 (ORISE Fellow)
Stefano Luccioli	HFS-200
Sylvester Mosley	HFS-255
Vladimir Yurovsky	HFS-255

Following introductions, FDA personnel opened the meeting by thanking the representatives of George Weston Foods, Ltd. for their submissions to FDA's Generally Recognized as Safe (GRAS) Notification Program. FDA personnel stated that one of the purposes of the GRAS Notification Program is to establish a dialogue between FDA and industry and that FDA appreciated the efforts of George Weston Foods, Ltd. staff in putting together their GRAS submissions.

FDA personnel then proceeded to explain why the notices failed to meet the standards for general recognition of safety. During the review process, FDA staff noted that it is well-established in the scientific literature that lupin proteins are similar enough to peanut proteins that peanut-allergic individuals may also react to lupin-containing products. Because of this cross-reactivity, peanut allergic individuals could potentially have life-threatening reactions when they eat lupin-containing products for the very first time. As there is little consumption of lupin-containing products in the United States, the peanut-allergic population is unaware of this danger. FDA staff stated that ingredient labels

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containing only the term, “sweet lupin” as proposed in the notices, would not be adequate to warn U.S. consumers of the risk of a reaction if they are peanut-allergic.

FDA personnel then covered the administrative options available under the GRAS Notification program for notices that fail to establish that an ingredient is generally recognized as safe for its intended uses. FDA personnel explained that any notifier may ask FDA to cease evaluation of a notice. In response to these requests, FDA posts an acknowledgement of the receipt of the request on FDA’s Internet site alongside the GRAS Notice number. In these cases, FDA’s acknowledgement uses standard language with no mention of the reasons why the notifier is requesting that FDA cease to evaluate the notice. Alternatively, FDA may issue a letter stating that FDA does not agree with the notifier’s conclusion that an ingredient is GRAS for its intended uses and post this letter on the FDA Internet site.


In discussing these administrative options, FDA personnel noted that the notices had been filed and assigned as GRN 000262, sweet lupin protein; GRN 000263, sweet lupin fiber; and GRN 000264, sweet lupin flour. FDA personnel went on to explain that George Weston Foods, Ltd. would not be receiving the standard acknowledgement letter that FDA sends out upon the filing of GRAS notices as FDA staff had difficulty determining appropriate names for the subject of GRN 000262. For its GRAS notice correspondence, FDA uses a technically descriptive name for the subject of the notice. In the case of GRN 000262, FDA reviewers were unable to determine enough of the details of the manufacturing process and subsequent physical/chemical characteristics of the subject to be able to assign a descriptive name.

FDA reviewers then briefly highlighted the technical issues that they noted during their reviews. Due to the detailed nature of these issues, FDA agreed to send a list of issues by electronic mail at a later date (electronic mail correspondence of February 23, 2009).


In concluding the discussion of the administrative details, FDA personnel stated that in their review of the lupin literature they had noticed that lupin flour may be yellow and used to mimic the addition of butter or eggs to baked goods. FDA personnel stated that under U.S. law, any new ingredient that is added to food with the intention of imparting color may be an unapproved color additive and may not be GRAS. The appropriate regulatory process in these instances is the color additive petition process.

Following the discussion with the review scientists, FDA personnel reiterated the main concern with the notices was the fact that a certain percentage of individuals who are allergic to peanuts will also be allergic to lupin. Given the lack of lupin consumption in the U.S. population, there is no awareness of this cross-reactivity among peanut allergic individuals and this presents a significant safety issue.

At the close of the meeting, FDA personnel and George Weston Foods, Ltd. representatives discussed administrative items, including the process for withdrawing a GRAS notice and the process for resubmitting a GRAS notice.

~~~~
Susan Carlson, Ph.D.

(b) (5)



000150

**George Weston Foods Limited**

ABN 45 008 429 632

SENT VIA INTERNATIONAL EXPRESS POST

December 10, 2008

Robert L. Martin, Ph.D.
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food And Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835


Re: Withdrawal of Generally Recognized As Safe (GRAS) Exemption Notices for Sweet Lupin Fiber, Sweet Lupin Flour, and Sweet Lupin Protein

Dear Dr. Martin:

This letter is to inform you that we would like to withdraw our GRAS Exemption Notices for Sweet Lupin Fiber, Sweet Lupin Flour, and Sweet Lupin Protein, which were forwarded to your office on September 26, 2008.

Thank you for your kind attention to this matter. Please contact me should you have any questions regarding the withdrawal of these Notices.

Sincerely,


Peter Schutz
Chief Executive

George Weston Technologies
A Division of George Weston Foods Limited
peter.schutz@gwf.com.au



000151

CORPORATE OFFICE

LEVEL 1 TOWER B 799 PACIFIC HIGHWAY CHATSWOOD NSW 2067
PO BOX 5579 WEST CHATSWOOD NSW 1515 AUSTRALIA
TELEPHONE +612 9415 1411 FACSIMILE +612 9419 2907

**Carlson, Susan**

From: Sherry.Duckworth@gwf.com.au
Sent: Monday, February 23, 2009 3:26 PM
To: Carlson, Susan
Subject: RE: Lupin GRAS Notices - reviewer's comments

Attachments: lupin comments.doc



lupin comments.doc
(62 KB)

Dear Susan

Thank you for collating the comments from the reviewers. Our team will review them and let you know if we have any questions. We would certainly meet with you prior to submitting a new GRAS Notice.

Regards
Sherry

"Carlson, Susan"
<Susan.Carlson@fd
a.hhs.gov>

24/02/2009 06:46
AM

To
<Sherry.Duckworth@gwf.com.au>
cc
Subject
RE: Lupin GRAS Notices - reviewer's
comments

Dear Sherry,

Here are the collated comments from our reviewers that were raised in our teleconference with you on December 4, 2008. As we stated at the meeting, given the complexities of these notices, we would strongly encourage you to meet with us prior to making any new GRAS Notice submissions for lupin.

My apologies for the delay. Thank you for your patience. Please don't hesitate to contact us if you have any questions.

Regards,
Susan

-----Original Message-----

From: Sherry.Duckworth@gwf.com.au [mailto:Sherry.Duckworth@gwf.com.au]
Sent: Friday, February 20, 2009 6:37 AM
To: Carlson, Susan

000157

Subject: RE: Lupin GRAS Notices - reviewer's comments

Dear Susan

Have you had any success in obtaining the comments from the supervisor?

Thanks
Sherry

"Carlson, Susan"

<Susan.Carlson@fd

a.hhs.gov>

To

<Sherry.Duckworth@gwf.com.au>

24/01/2009 08:32

cc

AM

Subject

reviewer's

RE: Lupin GRAS Notices -

comments

Hello Sherry,
Thank you for inquiring. The comments are with one of the supervisors. I
will gently remind him.
Thank you for your continued patience.
--Susan

-----Original Message-----

From: Sherry.Duckworth@gwf.com.au [mailto:Sherry.Duckworth@gwf.com.au]
Sent: Friday, January 23, 2009 4:31 PM
To: Carlson, Susan
Subject: Lupin GRAS Notices - reviewer's comments

Dear Susan

Just wondering how you are progressing with compiling the reviewer's
comments.

Thanks

Sherry

Sherry Duckworth
Project Manager
Research and Technology
George Weston Technologies
(A Division of George Weston Foods)
1 Braidwood Street
Enfield NSW 2136
Australia
Tel: + 61 2 9764 8160
Fax: + 61 2 9742 5959
MOB: +61 0419 412 398
Email: sherry_duckworth@gwf.com.au
----- Forwarded by Sherry Duckworth/WT/NSW/GWF on 24/01/2009 08:11 AM

Sherry

Duckworth/WT/NSW/

GWF

To

"Carlson, Susan"

21/12/2008 06:17

<Susan.Carlson@fda.hhs.gov>

PM

cc

Subject

reviewer's

Re: Lupin GRAS Notices -

comments(Document link: Sherry

Duckworth)

Thanks Susan. We will look forward to receiving them.

"Carlson, Susan"

<Susan.Carlson@fd

a.hhs.gov>

To

<Sherry.Duckworth@gwf.com.au>

20/12/2008 09:48

CC

AM

Subject

Lupin GRAS Notices - reviewer's
comments

Dear Sherry,

We are still trying to get our reviewers' comments together for you. The reviewers are working on them (and they are around for at least Monday and Tuesday of this coming week). We hope to get them to you soon. Thank you for your continued patience.

Regards,
Susan

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(See attached file: lupin comments.doc)

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KEY:

GRN 000262 Lupin Protein

GRN 000263 Lupin Fiber

GRN 000264 Lupin Flour

All review team members discussed the issue of cross-reactivity between lupin protein allergens and allergens in peanuts. Review team members noted that lupin protein is present in the flour and fiber ingredients (the subjects of GRN 000264 and GRN 000263) in addition to the protein ingredient (GRN 000262). The review team believes that this cross-reactivity is a safety issue for peanut allergic individuals in the U.S. who have no experience with lupin products and would have no way of knowing that they could already be sensitized to lupin and thus at risk.

The chemistry reviewers listed the following concerns from the notices:

- For GRN 000262, the identity section does not cover the following—
 - Protein name, molecular weight, sequence information.
 - The active component of the product (globulin or albumin?).
- For GRN 262 the following details are not clearly stated in the method of manufacture—
 - The chemical identity of Fractions 1 and 2, i.e. name of the protein in these fractions (alpha, beta, gamma conglutin). (Gamma conglutin has only been mentioned in the acute tox section (Page 15)).
 - The adjusted pH value or range (alkaline or acidic).
- Identities of the sweet varieties of the four species of sweet lupin that are used as source materials are unclear. What quality control procedures are used in the method of manufacture?
- The manufacturing processes described for GRN 000262 and GRN 000264 would not be expected to remove glycoalkaloids, mycotoxins, or saponins. Is there a preliminary soaking or boiling step?
- Maximum alkaloid and phomopsin levels in lupin flour (<200mg/kg and 5 mcg/kg, respectively), as set forth in GRN 000264, are the same as those of the Advisory Committee on Novel Foods and Processes (ACNFP) of the United Kingdom, (UK) and in the Australia New Zealand Food Standards Code. These levels appear to have been set based on limited information about exposure and toxicity of these natural toxicants. The notifier should address the following in a discussion of the general recognition of safety of intended uses of lupin ingredients:
 - Estimated cumulative intakes of sweet lupin from the three ingredients (flour, fiber, protein) are 92.6 g/p/d at the mean and 158.5 g/p/d at the 90th percentile. These intakes, assuming a 200 mg/kg glycoalkaloid level in sweet lupin and 70 kg human, are associated with glycoalkaloid exposures as high as 265 mcg/kg/d and 453 mcg/kg/d at the 90th percentile. These alkaloid exposures would be much greater than the tolerable exposure level of 35 mcg/kg/d tentatively established by ANZFA (2001) (cited on p. 000043 of GRN 000264.) A similar calculation can be performed for phomopsins, although no tolerable level has been set.

- The proposed food ingredient uses of lupin (as flour, fiber, protein) are more extensive than those currently reported in Europe and considered in the FSANZ review. For example, in GRN 000264, proposed use level (up to 25% replacement of other sources of flour)--exceeds that considered by Australia New Zealand Food Authority (ANZFA) (up to 10%); proposed level may be above realistic use level.
- For GRN 000262, the specifications section does not address the following –
 - microbiological specifications for consistency lots.
- For GRN 000264, batch analyses show yeast and mold specification not consistently met (p. 000113).
- For GRN 000264, one of the references (Bradbury et al., 2004) is confidential.
- For GRN 000264, we have noticed that due to the presence of carotenoids, lupin flour may be used to mimic the addition of eggs. Under U.S. law, this use would likely be regulated as a color additive, requiring the submission of a color additive petition.
- The detection method used to determine the level of phomopsins needs to be discussed. Also, the level of exposure is not well-explained.
- In the discussion of pesticide residue analysis, the notifier mentions several MRL standards for various grains. Further, this is not a comprehensive list of pesticides with MRLs for grains or for lupin. The notifier should clearly explain the justification for choosing these comparison standards and specific pesticides for analysis. Further, the Limit of Detection (LOD) values for certain pesticides are above the Maximum Residue Levels (MRL) set by FSANZ in 2005. The notifier cannot state that pesticides are present at or below the MRL. In a GRAS submission, we would expect reference to valid analytical methods with appropriate LODs.
- GRN 000262 does not include a discussion of the stability and metabolic fate of lupin protein. Such a discussion might include–
 - Protein digestibility with reference to the EFSA document that discusses possible resistance to digestion.
 - The heat stability of the protein.
- In GRN 000262, issues regarding Estimated Intake of Sweet Lupin Fractions 1 and 2 that should be addressed include–
 - Use of Fraction 1 in meat.
 - The use of lupin protein in infant formula (Page 13, Para 3).
 - Background protein consumption is 75.2 g (mean). In the expert panel opinion (Pg 3), the discussion of a conservative estimate of all uses + all users is 92.6 g. This implies that it is greater than the background (worst case scenario). Please clarify apparent inconsistency.
- In GRN 000262's 'Other Phytonutrient' section (Pg 23), there is a comparison made to soy in infant formula. If sweet lupin protein is intended to be used in infant formula, this should be clearly stated.

The toxicology reviewers then summarized the highlights of their concerns:

- For sweet lupin flour (GRN 000264), the test substance used in most of the safety studies cited in the notice were whole lupin seeds or lupin protein. There were three studies summarized in the notice on flour from individual lupin species. There was no study in the notice that used lupin flour derived from a mix of lupin species and varieties which is the subject of GRN 000264. In order for the studies to support the safe use of sweet lupin flour, the test article of the studies must be similar to the subject of the GRAS notice.
- The subchronic rat study (using *L. angustifolia* flour) had no concurrent controls and the data were compared with historical values from another laboratory. There were two human studies using *L. albus* flour. One study gave one (150 g) cookie/day enriched with lupin flour to subjects for 60 days and found no significant adverse effect. The second study, in men only, reported an average nitrogen digestibility level of 77% and an increase in plasma urea nitrogen.
- For GRN 000264, lupin is reported to be low in lysine, methionine, cysteine and calcium. George Weston Foods, Ltd. needs to state clearly the levels of these amino acids and calcium in the flour.
- For GRN 000264, *Lupinus albus* seed is reported to be very high in manganese and may be toxic. This was not addressed in the notice.
- For GRN 000262 and GRN 000263 phomopsin levels are not presented in the batch analyses presented in Appendix B-2. Moreover, the notifier did not provide the correct reference to the maximal established level of 5 ppb. The FSANZ document that the notifier refers to (http://www.foodstandards.gov.au/_srcfiles/TR1.pdf), states that in the absence of human data and in the absence of a NOEL in animals, it is not possible to derive a tolerable level for human exposure.
- Also for GRN 000262 and GRN 000263, more sensitive methods should be used to determine the levels of pesticide residues so that the established MRL can be met. Their levels may be <0.05 ppm as detected, but >0.02 ppm as established by FSANZ (Table B-3-1).
- For GRN 000263, subchronic toxicity data on lupin flour cannot support the safety of lupin hull fiber.
- For GRN 000262, the reviewer noted some minor issues. In Table 4 footnote b is missing. On P. 24 line 2, maybe 1.4 mg trypsin inhibitors/day? In Appendix B-4, Table B-3-1, why does the amount of insoluble fiber decrease during storage?

MEMORANDUM OF MEETING

Date: August 26, 2009

Time: 2:00 p.m. – 2:30 p.m.

Place: FDA, Center for Food Safety and Applied Nutrition, Office of Food Additive Safety, 4300 River Road, College Park, MD 20740, room 2073

Subject: GRN 000262, sweet lupin protein; GRN 000263 sweet lupin fiber; GRN 000264, sweet lupin flour

Participants:Visitor:

Melody Harwood

Cantox Health Sciences International

FDA:

Susan Carlson

HFS-255

Robert Martin

HFS-255

The meeting was held at the request of Ms. Harwood on behalf of Cantox's client, George Weston Foods, Ltd., for the purpose of discussing the steps needed to resubmit the previously withdrawn GRAS Notices for sweet lupin protein, sweet lupin fiber, and sweet lupin flour (Attachment). Ms. Harwood stated that George Weston Foods, Ltd. wished to pursue labeling as a condition of safe use for their lupin-based ingredients.


FDA personnel noted that because it has been established in the scientific literature that peanut-allergic individuals also react to lupin-based ingredients we believe it will be difficult to establish a basis for the general recognition of safety for lupin, even with a label statement.

FDA personnel and Ms. Harwood discussed the possibility of submitting food additive petitions instead of GRAS Notices. FDA personnel cautioned Ms. Harwood that given the well-known cross-reactivity of lupin proteins and peanut proteins and the lack of consumer awareness in the U.S. market, it would be difficult to establish the safe use of lupin ingredients, even with a regulation.

Ms. Harwood thanked FDA personnel for their time and stated that she would convey the information to George Weston Foods, Ltd.


Susan Carlson, Ph.D.

Attachment

(b) (5)


000165

Attachment

From: Melody Harwood
To: Carlson, Susan;
cc: Martin, Robert L;
Subject: RE: Request for Meeting on Lupin October 30, 2009
Date: Monday, August 24, 2009 3:45:45 PM

OK, sounds great. Thanks for your consideration. I look forward to seeing you on Wednesday.

Kind regards,
Melody

-----Original Message-----

From: Carlson, Susan [<mailto:Susan.Carlson@fda.hhs.gov>]
Sent: Monday, August 24, 2009 1:48 PM
To: Melody Harwood
Cc: Martin, Robert L
Subject: RE: Request for Meeting on Lupin October 30, 2009

Dear Melody,
Please plan on spending a few minutes (5 to 10 min) with Dr. Martin and myself following your meeting here Wednesday, August 26. We would like to discuss our current thinking on the allergenicity issues of lupin.
Thank you,
Susan

-----Original Message-----

From: Melody Harwood [<mailto:mharwood@cantox.com>]
Sent: Monday, August 24, 2009 12:01 PM
To: Carlson, Susan
Cc: Ashley Roberts
Subject: Request for Meeting on Lupin October 30, 2009

Hi Dr. Carlson,

I hope this finds you well and enjoying summer time in Maryland.

I am contacting you to request a meeting with our client, George Weston Foods, Australia, to discuss some of the issues that were identified by the agency during their review last fall of 3 GRAS Exemption Claims for their lupin ingredients. In particular, the current issue that our client is facing pertains to potential allergenicity of the lupin

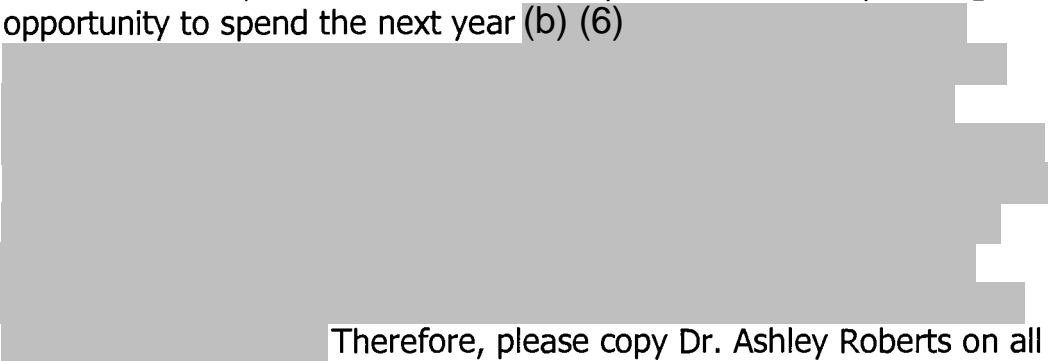
000166

proteins, as well as potential cross-reactivity of these proteins with other allergens (including peanut) and how to adequately label retail products containing these ingredients so as to properly inform any lupin- or peanut-sensitive individuals.

Our client has been working with several experts, including Dr. Steve Taylor, various clinicians, and members of Cantox, and would like to request a meeting with CFSAN to discuss this matter with all that need to be involved. It is requested that representatives from both OFAS and ONLDS be invited to attend this meeting, and I want to get your opinion if we should also invite a representative of the Office of Regulations, Policy and Social Sciences or of the Office of Compliance, as I believe this is an unprecedented issue? Perhaps even Michael Landa, Deputy Director for Regulatory Affairs, should be included? Our client will be coming from Australia and have already confirmed the availability of their other experts for October 30, 2009. I do realize that you usually need a couple of suggested days in order to find a date that suits the schedules of all involved, but I'm hoping that providing ample time before the meeting will allow for this date to be feasible to meet.

I look forward to your thoughts on this matter. I will be down in College Park on Wednesday, August 26th, and if there is an opportunity to discuss a meeting strategy with you and your colleagues I would be grateful and will gladly make myself available after 2 pm.

On another note, I am excited to inform you that I will be pursuing an opportunity to spend the next year (b) (6)



Therefore, please copy Dr. Ashley Roberts on all correspondence related to this meeting request, as he will continue to work with yourself and our client subsequent to my last day at Cantox to finalize a meeting date..

Please don't hesitate to let me know if you have any questions or concerns.

Kind regards,
Melody

Melody Harwood, M.Sc.
Associate Director, Business Development
Food and Nutrition
Cantox Health Sciences International

2233 Argentia Road, Suite 308
Mississauga, ON, L5N 2X7, CANADA
Tel: 905-542-2900, Extension 302
Fax: 905-542-1011
Mobile: 905-580-6693
mharwood@cantox.com

The 4th Practical Short Course on Functional Oils: Omega-3 Fatty Acids
Market Trends, Nutrition & Health, Utilization in Food Systems is August
24-25, 2009 in Chicago, IL! Ms. Lina Paulionis of Cantox will be
presenting, "Omega-3 Oils: Health Claims Global Perspectives" on August
24 at 10:00 a.m. Don't miss it!
<<http://home.scarlet.be/~tpm12374/smartshortcourses/pdf/4thFLipids.pdf>>
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000168

Carlson, Susan

SU

**From:** Kane, Rhonda R.**Sent:** Thursday, October 08, 2009 9:45 AM**To:** Billingslea, Felicia B; June, Geraldine A; Martin, Robert L; Carlson, Susan; Dinovi, Michael J; Luccioli, Stefano; Tarantino, Laura M**Subject:** RE: Outstanding Industry Issues About Lupine

To all,

[REDACTED] ([REDACTED]
[REDACTED] b [REDACTED]
[REDACTED]) [REDACTED]
[REDACTED] ([REDACTED]
[REDACTED] 5 [REDACTED]
[REDACTED]) [REDACTED]
[REDACTED]

Rhonda R. Kane, MS, RD
Consumer Safety Officer (HFS-820)
Food Labeling and Standards Staff
ONLDS / CFSAN / FDA
CP 1, Room 4D-008
Phone: (301) 436-1803
Fax: (301) 436-2636
E-mail: Rhonda.Kane@fda.hhs.gov

From: Carlson, Susan**Sent:** Monday, October 05, 2009 3:09 PM**To:** Kane, Rhonda R.**Cc:** Billingslea, Felicia B; June, Geraldine A; Martin, Robert L**Subject:** RE: Outstanding Industry Issues About Lupin

Dear Rhonda,

(b) (5) [REDACTED]
[REDACTED]
[REDACTED]

Thank you Rhonda,
--Susan

From: Martin, Robert L**Sent:** Tuesday, September 29, 2009 7:42 AM**To:** Carlson, Susan**Cc:** Billingslea, Felicia B; June, Geraldine A; Kane, Rhonda R.**Subject:** RE: Outstanding Industry Issues About Lupin

000169

(b) (5)

Thanks.

Robert L. Martin

301-436-1219

From: Carlson, Susan

Sent: Monday, September 28, 2009 3:15 PM

To: Martin, Robert L

Cc: Billingslea, Felicia B; June, Geraldine A; Kane, Rhonda R.

Subject: FW: Outstanding Industry Issues About Lupin

Hello Bob,

(b) (5)

Thank you,
Susan

From: Kane, Rhonda R.

Sent: Monday, September 28, 2009 2:26 PM

To: Carlson, Susan

Subject: FW: Outstanding Industry Issues About Lupin

FYI

From: Dinovi, Michael J

Sent: Monday, September 28, 2009 12:54 PM

To: Kane, Rhonda R.

Subject: RE: Outstanding Industry Issues About Lupin

Rhonda

000170

(b) (5)

Mike

From: Kane, Rhonda R.
Sent: Monday, September 28, 2009 12:31 PM
To: Dinovi, Michael J
Subject: Outstanding Industry Issues About Lupin

Hi, Michael,

(b) (5)

Rhonda R. Kane, MS, RD
Consumer Safety Officer (HFS-820)
Food Labeling and Standards Staff
ONLDS / CFSAN / FDA
CP 1, Room 4D-008
Phone: (301) 436-1803
Fax: (301) 436-2636
E-mail: Rhonda.Kane@fda.hhs.gov

From: Billingslea, Felicia B
Sent: Monday, September 28, 2009 10:44 AM
To: 'Ashley Roberts'; Tarantino, Laura M
Cc: Ian Munro
Subject: RE: Meeting on Lupin

Hi Ashley,

I am aware of your requests and will ask one of my staff to follow up with you.

Thanks,
Felicia

From: Ashley Roberts [<mailto:aroberts@cantox.com>]
Sent: Wednesday, September 23, 2009 8:11 AM
To: Tarantino, Laura M
Cc: Ian Munro; Billingslea, Felicia B
Subject: RE: Meeting on Lupin

Dear Laura,

Many Thanks for your quick response and advice.

I will make contact with Felicia directly on this matter

Kind Regards

Ashley

000171

From: Tarantino, Laura M [mailto:Laura.Tarantino@fda.hhs.gov]
Sent: Wednesday, September 23, 2009 8:03 AM
To: Ashley Roberts
Cc: Ian Munro; Billingslea, Felicia B
Subject: RE: Meeting on Lupin

Hi, Ashley,

We had suggested that your initial contact be Felicia Billingslea in our labeling group, since the specific questions are primarily about labeling precedents. She or one of her staff will help arrange the meeting, and will keep Dr. Carlson and others in OFAS in the loop.

Laura Tarantino

From: Ashley Roberts [mailto:aroberts@cantox.com]
Sent: Tuesday, September 22, 2009 4:28 PM
To: Tarantino, Laura M
Cc: Ian Munro
Subject: FW: Meeting on Lupin

Dear Dr. Tarantino,

I just wanted to follow-up on some previous correspondence between Dr. Munro and yourself regarding the setting up of a meeting between the Agency, the manufacturer of lupin and Dr. Steve Taylor. Unfortunately, Dr. Munro has been out of the office recently and he has asked me to make contact with you again regarding this matter.

Dr. Munro has informed me that you recommended that we should try to set up a meeting by speaking firstly with one of your staff members whose name we are now seeking. I am led to believe that this person was not Dr. Carlson as outlined below. Please could you relay to me who this person was so that I might speak with them directly on this matter.

I look forward to receiving your response on this matter

Kind Regards

Ashley Roberts, PhD
VP, Food & Nutrition Group

CANTOX Health Sciences International
2233 Argentia Road, Suite 308
Mississauga, Ontario, Canada
L5N 2X7

T: 905-542-2900
F: 905-542-1011
E: aroberts@cantox.com
W: www.cantox.com

From: Ian Munro
Sent: Tuesday, July 14, 2009 2:17 PM
To: Tarantino, Laura M
Subject: RE: Meeting on Lupin

Hi Laura....Thanks for your email. No I am nor in Aspen this week but stuck working in Toronto. I have a hectic week this week but I would like to call you next week after Monday. So why don't I shoot you an email early next week and you can tell me a good time to call.....Thanks.....Ian

Ian C. Munro, Ph.D., F.A.T.S., FRCPath
Executive Vice President

000172

Senior Scientific Consultant
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imunro@cantox.com
www.cantox.com



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From: Tarantino, Laura M [<mailto:Laura.Tarantino@fda.hhs.gov>]
Sent: Monday, July 13, 2009 4:10 PM
To: Ian Munro
Subject: RE: Meeting on Lupin

Hi, Ian,

Thank you so much for this background. It is very helpful. And I am sorry for my delay in responding, but I was on leave until last week, and I am just now catching up on email. Before I left, I did talk to a few folks, so I think we can work on setting up a meeting, and your notion of contacting Susan Carlson to coordinate is a good one. However, I'd be happy to discuss with you beforehand, if it will be helpful.

I am assuming you are in Aspen this week. I am not in College Park today, but should be there the rest of this week and most of next. I will plan to try to contact you early next week, or give me a holler before then if convenient.

Laura

From: Ian Munro [<mailto:imunro@cantox.com>]
Sent: Monday, June 29, 2009 10:26 AM
To: Tarantino, Laura M
Cc: Melody Harwood
Subject: RE: Meeting on Lupin

Laura,

Many thanks for your help so far. Here is a brief synopsis of what has happened to date.

We had a pre-submission meeting on September 10, 2007 with several OFAS people.

- Dr. Michael DiNovi, OFAS/DBGNR
- Dr. Ron Chanderbhan, OFAS/DBGNR
- Dr. Paulette Gaynor, OFAS/DBGNR
- Dr. Marcella Fruchter, OFDCER/DEC/COCB
- Dr. Jeremy Mihalov, OFAS/DBGNR

There didn't seem to be any concerns about the overall safety of lupin ingredients including lupin protein, flour and fibre until the potential allergenicity of lupin protein was mentioned, and even more so when the potential cross-reactivity with peanut was disclosed. Coming out of that meeting, it was recommended that we speak with a representative of the Office of Nutrition, Labelling and Dietary Supplements (ONLDS), so one of our people, Melody Harwood called and spoke with Rhonda Kane and explained the situation to her. As there currently isn't a policy for labelling for 'new' or cross-reactive allergens, she was not able to give clear guidance on what to do other than to comply with the ingredient labelling requirements, as per the regulations.

000173

We submitted the Notices for all three ingredients and yes, there were questions raised during the initial review phase (prior to withdrawal as a consequence of the allergen question), but none that were considered to be insurmountable. Our client indicated that the general feel they got in subsequent

discussions with OFAS was that the concerns of potential allergenicity and cross-reactivity were paramount and that the issue would not go away with simple conformity to the current ingredient and allergen labelling requirements, as these would not provide an avenue for warning or decreasing risk in lupin- or peanut-allergic individuals.

I believe that Dr. Susan Carlson handled the client's initial submission, and so we have planned to contact her to set up the meeting once we have settle on who should attend from your side

Thanks for your help.

Ian

From: Tarantino, Laura M [mailto:Laura.Tarantino@fda.hhs.gov]

Sent: Tuesday, June 16, 2009 3:22 PM

To: Ian Munro

Subject: RE: Meeting with CFSAN

Hello, Ian,

I did hear your voicemail. I have been, and am still today, away from my own office and travelling among buildings. I will give you a call back this week. I appreciate your wanting to address the cross-reactivity concern and willingness to work out something on labelling. Of course we will be happy to meet. We will have some discussion about who to have from our end. In the meantime, do let's reconnect later this week.

Best regards,

Laura

From: Ian Munro [mailto:imunro@cantox.com]

Sent: Tuesday, June 16, 2009 12:34 PM

To: Tarantino, Laura M

Subject: FW: Meeting with CFSAN

Dear Laura,

As per my voice mail of yesterday, I wanted to speak with you/get your feedback on the logistics of a meeting to discuss the labelling and self-affirmed GRAS status of our client's various lupin-derived ingredients.

These ingredients have been sa-GRAS, and the current issue that our client is facing pertains to potential allergenicity of the lupin proteins, as well as potential cross-reactivity of these proteins with peanut and how to adequately label retail products containing these ingredients so as to properly inform any lupin- or peanut-sensitive individuals. We have in the past discussed this concern with representatives of both the Office of Food Additive Safety and of the Office of Nutrition, Labeling and Dietary Supplements of CFSAN; however, this appears to be a situation without precedent under CFSAN policy and so a solution has not yet been reached.

Our client has been working with several experts, including Dr. Steve Taylor and various clinicians, as well as members of my staff, and would like to request a meeting with CFSAN to discuss this matter with all involved. It is anticipated that representatives from both OFAS and ONLDS would be invited to attend this meeting, and I want to get your opinion if we should also invite a representative of the Office of Regulations, Policy and Social Sciences or of the Office of Compliance. Perhaps even Michael Landa, Deputy Director for Regulatory Affairs, should be included?

I look forward to hearing from you at your earliest convenience. Many thanks in advance for your assistance with this matter.

Kind regards,

Ian

Ian C. Munro, Ph.D., F.A.T.S., FRCPATH
Executive Vice President
Senior Scientific Consultant
Cantox Health Sciences International
2233 Argentia Road, Suite 308
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Fax: (905) 542-1011
imunro@cantox.com
www.cantox.com



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Are you attending NutrEvent in Lille, France, June 17-18, 2009?

Cantox's **Nigel Baldwin** will be speaking & participating in the Roundtable Discussion during the Regulatory Frameworks Seminar, June 17.

Memorandum of Meeting

PR



Date: September 10, 2007
Time: 10:00 - 11:00 a.m.
Place: University Station, 4300 River Road, Room 2013
Subject: Pre-submission meeting for lupin derived ingredients

Participants:Industry:

Joseph F. Borzelleca	Medical College of Virginia
Fiona Fleming	George Weston Foods
Melody Harwood	Cantox

FDA:

Ronald Chanderbhan	HFS-255
Michael DiNovi	HFS-255
Marcella Fruchter	HFS-255
Paulette Gaynor	HFS-255
Jeremy Mihalov	HFS-255

Ms. Melody Harwood requested the meeting to discuss submitting a notice in accordance with the Food and Drug Administration's proposed regulation, proposed 21 CFR 170.36 (62 FR 18938; April 17, 1997; Substances Generally Recognized as Safe (GRAS). The visitors presented an overview of the company (George Weston Foods/Weston Technologies) and of the data and information that the company is relying on to support its view that the intended use of the six lupin derived ingredients (various types of flour, proteins, or fibers) are GRAS.

FDA representatives inquired whether the visitors knew what alkaloids would be present in the ingredients, reminding them to include that information in the submission. We also asked whether the ingredient would be used in meat and poultry products because such uses would result in an USDA review, suggesting the visitors check with USDA for any specifics that they need for their review. FDA representatives suggested separating the six ingredients into more than one submission.

The visitors stated that lupin-derived ingredients may cross react with peanuts. The visitors also stated that a recent EFSA opinion amended the list of known allergens to include lupin, suggesting that the United States could do the same, identifying the source as is done with soy, for example. FDA representatives stated that because of the cross-reactivity to peanut, people will be concerned about these ingredients. In response to the visitors question as to how protein cross-reactivity is handled, we offered to provide a contact for the FALCPA group. Subsequent to the meeting, we provided contact information for Felicia Billingslea.

Paulette Gaynor, Ph.D.

(b) (5)

FILE
COPY

OFFICE	SURNAME	DATE	OFFICE	SURNAME	DATE	OFFICE	SURNAME	DATE
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PRE-NOTIFICATION MEETING FOR LUPIN-DERIVED INGREDIENTS

Monday, September 10, 2007 at 10 a.m.

**4300 River Rd., Room 2073
College Park, MD 20740**

Participants: CFSAN Representatives
Ms. Fiona Fleming, George Weston Foods
Dr. Joseph F. Borzelleca, Medical College of Virginia
Ms. Melody Harwood, Cantox Health Sciences International

- 1. Introductions**
- 2. Overview of George Weston Foods / Weston Technologies**
- 3. Basis for GRAS**
 - (a) Manufacturing, Chemistry, Specifications**
 - (b) Uses and Exposure**
 - (c) Data Supporting GRAS status**
- 4. Next Steps**

Gaynor, Paulette M

From: Gaynor, Paulette M
Sent: Monday, July 16, 2007 3:35 PM
To: Fruchter, Marcella I
Subject: FW: Request for Pre-Notification Meeting - Lupin-derived ingredients
Attachments: Draft FDA Meeting Agenda.doc

Marcella,

Please serve as CSO for this meeting.

Thank you,

Paulette

From: Martin, Robert L
Sent: Monday, July 16, 2007 7:26 AM
To: 'Melody Harwood'; Gaynor, Paulette M
Subject: FW: Request for Pre-Notification Meeting - Lupin-derived ingredients

Ms. Harwood, by way of this e-mail message, I am forwarding your request for a pre-submission meeting to Dr. Paulette Gaynor who will assign it to someone in her group to contact you and arrange this meeting. Someone from her group will be contacting you soon.

Thanks.
Robert L. Martin
301-436-1219

From: Melody Harwood [mailto:mharwood@cantox.com]
Sent: Monday, July 16, 2007 1:13 AM
To: Martin, Robert L
Subject: Request for Pre-Notification Meeting - Lupin-derived ingredients

Dear Dr. Martin,

I am contacting you on behalf of our client, Weston Technologies, a division of George Weston Foods Limited, who would like to schedule a meeting with representatives of the Administration to go over the self-affirmation of the Generally Recognized As Safe (GRAS) status of their lupin-derived food ingredients prior to submitting a GRAS Notification. Below, please find information that might be useful for scheduling the meeting.

- Name of Company: Weston Technologies, a division of George Weston Foods Limited, Enfield, Australia
- Attendees: Ms. Fiona Fleming (Weston Technologies), Dr. Steve Taylor (University of Nebraska), and myself, Melody Harwood (CANTOX)

- The products are derived from sweet varieties of *Lupinus* spp. (lupin) (*L. angustifolia*, *L. albus*, *L. luteus*, and *L. mutabilis*) and include lupin flour, two lupin protein fractions, two lupin kernel fibres, and lupin hull fibre.
- The lupin-derived food ingredients will be marketed for use in various traditional food products such as bakery products, breakfast cereals, and beverages.
- The objectives of the meeting are to provide an overview of the company and the basis for GRAS of the lupin-derived ingredients under the intended conditions of use.
- Equipment needs: In-focus or other projection machine to connect to a laptop for visual presentation

A draft agenda is attached for your review. I would like to propose a meeting on September 10 or 11, 2007, as our client is in North America from Australia for a short period of time during September and unfortunately, these dates are the only ones that seems to fit her schedule. I do hope that it also is suitable for yourself and representatives of your department. If not, please let me know what alternate dates would be convenient, and perhaps I can re-arrange my schedule to accommodate.

<<Draft FDA Meeting Agenda.doc>>

I would like to thank you in advance for your assistance in this matter. If you require further information, please do not hesitate to contact my by telephone or via email. I look forward to hearing from you at your earliest convenience.

Kindest regards,

Melody

Melody Harwood, M.Sc.
Associate Director, Business Development
Food and Nutrition
CANTOX HEALTH SCIENCES INTERNATIONAL

2233 Argentia Road, Suite 308
Mississauga, ON L5N 2X7 CANADA
Tel: 905-542-2900, extension 302
Fax: 905-542-1011
Mobile: 905-580-6693
mharwood@cantox.com

PRE-NOTIFICATION MEETING FOR LUPIN-DERIVED INGREDIENTS

DRAFT AGENDA

September 10 or 11, 2007

**4300 River Rd.
College Park, MD 20740**

Participants: CFSAN Representatives - To be Determined
Ms. Fiona Fleming, Weston Technologies
Dr. Steve Taylor, University of Nebraska
Ms. Melody Harwood, CANTOX Health Sciences International

- 1. Introductions**
- 2. Overview of Weston Technologies/George Weston Foods**
- 3. Basis for GRAS**
 - (a) Manufacturing, Chemistry, Specifications**
 - (b) Uses and Exposure**
 - (c) Data Supporting GRAS status**
- 4. Next Steps**

MEMORANDUM OF TELECONFERENCE

Date: December 4, 2008

Time: 2:00 p.m. – 2:45 p.m.

Place: FDA, Center for Food Safety and Applied Nutrition, Office of Food Additive Safety, 4300 River Road, College Park, MD 20740

Subject: GRN 000262, sweet lupin protein; GRN 000263 sweet lupin fiber; GRN 000264, sweet lupin flour

Participants:Telephone:

Sherry Duckworth	George Weston Foods, Ltd.
Cathy Fryirs	George Weston Foods, Ltd.
Fiona Fleming	George Weston Foods, Ltd.

FDA:

Susan Carlson	HFS-255
Ronald Chanderbhan	HFS-255
Michael DiNovi	HFS-255
Bianca Farias	HFS-255 (ORISE Fellow)
Paulette Gaynor	HFS-255
Molly Harry	HFS-255 (ORISE Fellow)
Stefano Luccioli	HFS-200
Sylvester Mosley	HFS-255
Vladimir Yurovsky	HFS-255

Following introductions, FDA personnel opened the meeting by thanking the representatives of George Weston Foods, Ltd. for their submissions to FDA's Generally Recognized as Safe (GRAS) Notification Program. FDA personnel stated that one of the purposes of the GRAS Notification Program is to establish a dialogue between FDA and industry and that FDA appreciated the efforts of George Weston Foods, Ltd. staff in putting together their GRAS submissions.

FDA personnel then proceeded to explain why the notices failed to meet the standards for general recognition of safety. During the review process, FDA staff noted that it is well-established in the scientific literature that lupin proteins are similar enough to peanut proteins that peanut-allergic individuals may also react to lupin-containing products. Because of this cross-reactivity, peanut allergic individuals could potentially have life-threatening reactions when they eat lupin-containing products for the very first time. As there is little consumption of lupin-containing products in the United States, the peanut-allergic population is unaware of this danger. FDA staff stated that ingredient labels

000148

containing only the term, “sweet lupin” as proposed in the notices, would not be adequate to warn U.S. consumers of the risk of a reaction if they are peanut-allergic.

FDA personnel then covered the administrative options available under the GRAS Notification program for notices that fail to establish that an ingredient is generally recognized as safe for its intended uses. FDA personnel explained that any notifier may ask FDA to cease evaluation of a notice. In response to these requests, FDA posts an acknowledgement of the receipt of the request on FDA’s Internet site alongside the GRAS Notice number. In these cases, FDA’s acknowledgement uses standard language with no mention of the reasons why the notifier is requesting that FDA cease to evaluate the notice. Alternatively, FDA may issue a letter stating that FDA does not agree with the notifier’s conclusion that an ingredient is GRAS for its intended uses and post this letter on the FDA Internet site.

In discussing these administrative options, FDA personnel noted that the notices had been filed and assigned as GRN 000262, sweet lupin protein; GRN 000263, sweet lupin fiber; and GRN 000264, sweet lupin flour. FDA personnel went on to explain that George Weston Foods, Ltd. would not be receiving the standard acknowledgement letter that FDA sends out upon the filing of GRAS notices as FDA staff had difficulty determining appropriate names for the subject of GRN 000262. For its GRAS notice correspondence, FDA uses a technically descriptive name for the subject of the notice. In the case of GRN 000262, FDA reviewers were unable to determine enough of the details of the manufacturing process and subsequent physical/chemical characteristics of the subject to be able to assign a descriptive name.

FDA reviewers then briefly highlighted the technical issues that they noted during their reviews. Due to the detailed nature of these issues, FDA agreed to send a list of issues by electronic mail at a later date (electronic mail correspondence of February 23, 2009).


In concluding the discussion of the administrative details, FDA personnel stated that in their review of the lupin literature they had noticed that lupin flour may be yellow and used to mimic the addition of butter or eggs to baked goods. FDA personnel stated that under U.S. law, any new ingredient that is added to food with the intention of imparting color may be an unapproved color additive and may not be GRAS. The appropriate regulatory process in these instances is the color additive petition process.

Following the discussion with the review scientists, FDA personnel reiterated the main concern with the notices was the fact that a certain percentage of individuals who are allergic to peanuts will also be allergic to lupin. Given the lack of lupin consumption in the U.S. population, there is no awareness of this cross-reactivity among peanut allergic individuals and this presents a significant safety issue.

At the close of the meeting, FDA personnel and George Weston Foods, Ltd. representatives discussed administrative items, including the process for withdrawing a GRAS notice and the process for resubmitting a GRAS notice.

~~~~
Susan Carlson, Ph.D.

(b) (5)



000150



Carlson, Susan

From: Sherry.Duckworth@gwf.com.au
Sent: Monday, February 23, 2009 3:26 PM
To: Carlson, Susan
Subject: RE: Lupin GRAS Notices - reviewer's comments

Attachments: lupin comments.doc



lupin comments.doc
(62 KB)

Dear Susan

Thank you for collating the comments from the reviewers. Our team will review them and let you know if we have any questions. We would certainly meet with you prior to submitting a new GRAS Notice.

Regards
Sherry

"Carlson, Susan"
<Susan.Carlson@fd
a.hhs.gov>

24/02/2009 06:46
AM

<Sherry.Duckworth@gwf.com.au>

To

cc

Subject
RE: Lupin GRAS Notices - reviewer's
comments

Dear Sherry,

Here are the collated comments from our reviewers that were raised in our teleconference with you on December 4, 2008. As we stated at the meeting, given the complexities of these notices, we would strongly encourage you to meet with us prior to making any new GRAS Notice submissions for lupin.

My apologies for the delay. Thank you for your patience. Please don't hesitate to contact us if you have any questions.

Regards,
Susan

-----Original Message-----

From: Sherry.Duckworth@gwf.com.au [mailto:Sherry.Duckworth@gwf.com.au]
Sent: Friday, February 20, 2009 6:37 AM
To: Carlson, Susan

000157

Subject: RE: Lupin GRAS Notices - reviewer's comments

Dear Susan

Have you had any success in obtaining the comments from the supervisor?

Thanks
Sherry

"Carlson, Susan"

<Susan.Carlson@fd

a.hhs.gov>

To

<Sherry.Duckworth@gwf.com.au>

24/01/2009 08:32

cc

AM

Subject

reviewer's

RE: Lupin GRAS Notices -
comments

Hello Sherry,
Thank you for inquiring. The comments are with one of the supervisors. I
will gently remind him.
Thank you for your continued patience.
--Susan

-----Original Message-----

From: Sherry.Duckworth@gwf.com.au [mailto:Sherry.Duckworth@gwf.com.au]
Sent: Friday, January 23, 2009 4:31 PM
To: Carlson, Susan
Subject: Lupin GRAS Notices - reviewer's comments

Dear Susan

Just wondering how you are progressing with compiling the reviewer's
comments.

Thanks

Sherry

Sherry Duckworth
Project Manager
Research and Technology
George Weston Technologies
(A Division of George Weston Foods)
1 Braidwood Street
Enfield NSW 2136
Australia
Tel: + 61 2 9764 8160
Fax: + 61 2 9742 5959
MOB: +61 0419 412 398
Email: sherry_duckworth@gwf.com.au
----- Forwarded by Sherry Duckworth/WT/NSW/GWF on 24/01/2009 08:11 AM

Sherry

Duckworth/WT/NSW/

GWF

To

"Carlson, Susan"

21/12/2008 06:17

<Susan.Carlson@fda.hhs.gov>

PM

cc

Subject

reviewer's

Re: Lupin GRAS Notices -

comments(Document link: Sherry

Duckworth)

Thanks Susan. We will look forward to receiving them.

"Carlson, Susan"

<Susan.Carlson@fd

a.hhs.gov>

To

<Sherry.Duckworth@gwf.com.au>

20/12/2008 09:48

CC

AM

Subject

Lupin GRAS Notices - reviewer's
comments

Dear Sherry,

We are still trying to get our reviewers' comments together for you. The reviewers are working on them (and they are around for at least Monday and Tuesday of this coming week). We hope to get them to you soon. Thank you for your continued patience.

Regards,
Susan

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(See attached file: lupin comments.doc)

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Any views or opinions expressed are those of the author and do not necessarily represent
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000161

KEY:

GRN 000262 Lupin Protein

GRN 000263 Lupin Fiber

GRN 000264 Lupin Flour

All review team members discussed the issue of cross-reactivity between lupin protein allergens and allergens in peanuts. Review team members noted that lupin protein is present in the flour and fiber ingredients (the subjects of GRN 000264 and GRN 000263) in addition to the protein ingredient (GRN 000262). The review team believes that this cross-reactivity is a safety issue for peanut allergic individuals in the U.S. who have no experience with lupin products and would have no way of knowing that they could already be sensitized to lupin and thus at risk.

The chemistry reviewers listed the following concerns from the notices:

- For GRN 000262, the identity section does not cover the following—
 - Protein name, molecular weight, sequence information.
 - The active component of the product (globulin or albumin?).
- For GRN 262 the following details are not clearly stated in the method of manufacture—
 - The chemical identity of Fractions 1 and 2, i.e. name of the protein in these fractions (alpha, beta, gamma conglutin). (Gamma conglutin has only been mentioned in the acute tox section (Page 15)).
 - The adjusted pH value or range (alkaline or acidic).
- Identities of the sweet varieties of the four species of sweet lupin that are used as source materials are unclear. What quality control procedures are used in the method of manufacture?
- The manufacturing processes described for GRN 000262 and GRN 000264 would not be expected to remove glycoalkaloids, mycotoxins, or saponins. Is there a preliminary soaking or boiling step?
- Maximum alkaloid and phomopsin levels in lupin flour (<200mg/kg and 5 mcg/kg, respectively), as set forth in GRN 000264, are the same as those of the Advisory Committee on Novel Foods and Processes (ACNFP) of the United Kingdom, (UK) and in the Australia New Zealand Food Standards Code. These levels appear to have been set based on limited information about exposure and toxicity of these natural toxicants. The notifier should address the following in a discussion of the general recognition of safety of intended uses of lupin ingredients:
 - Estimated cumulative intakes of sweet lupin from the three ingredients (flour, fiber, protein) are 92.6 g/p/d at the mean and 158.5 g/p/d at the 90th percentile. These intakes, assuming a 200 mg/kg glycoalkaloid level in sweet lupin and 70 kg human, are associated with glycoalkaloid exposures as high as 265 mcg/kg/d and 453 mcg/kg/d at the 90th percentile. These alkaloid exposures would be much greater than the tolerable exposure level of 35 mcg/kg/d tentatively established by ANZFA (2001) (cited on p. 000043 of GRN 000264.) A similar calculation can be performed for phomopsins, although no tolerable level has been set.

- The proposed food ingredient uses of lupin (as flour, fiber, protein) are more extensive than those currently reported in Europe and considered in the FSANZ review. For example, in GRN 000264, proposed use level (up to 25% replacement of other sources of flour)--exceeds that considered by Australia New Zealand Food Authority (ANZFA) (up to 10%); proposed level may be above realistic use level.
- For GRN 000262, the specifications section does not address the following –
 - microbiological specifications for consistency lots.
- For GRN 000264, batch analyses show yeast and mold specification not consistently met (p. 000113).
- For GRN 000264, one of the references (Bradbury et al., 2004) is confidential.
- For GRN 000264, we have noticed that due to the presence of carotenoids, lupin flour may be used to mimic the addition of eggs. Under U.S. law, this use would likely be regulated as a color additive, requiring the submission of a color additive petition.
- The detection method used to determine the level of phomopsins needs to be discussed. Also, the level of exposure is not well-explained.
- In the discussion of pesticide residue analysis, the notifier mentions several MRL standards for various grains. Further, this is not a comprehensive list of pesticides with MRLs for grains or for lupin. The notifier should clearly explain the justification for choosing these comparison standards and specific pesticides for analysis. Further, the Limit of Detection (LOD) values for certain pesticides are above the Maximum Residue Levels (MRL) set by FSANZ in 2005. The notifier cannot state that pesticides are present at or below the MRL. In a GRAS submission, we would expect reference to valid analytical methods with appropriate LODs.
- GRN 000262 does not include a discussion of the stability and metabolic fate of lupin protein. Such a discussion might include–
 - Protein digestibility with reference to the EFSA document that discusses possible resistance to digestion.
 - The heat stability of the protein.
- In GRN 000262, issues regarding Estimated Intake of Sweet Lupin Fractions 1 and 2 that should be addressed include–
 - Use of Fraction 1 in meat.
 - The use of lupin protein in infant formula (Page 13, Para 3).
 - Background protein consumption is 75.2 g (mean). In the expert panel opinion (Pg 3), the discussion of a conservative estimate of all uses + all users is 92.6 g. This implies that it is greater than the background (worst case scenario). Please clarify apparent inconsistency.
- In GRN 000262's 'Other Phytonutrient' section (Pg 23), there is a comparison made to soy in infant formula. If sweet lupin protein is intended to be used in infant formula, this should be clearly stated.

The toxicology reviewers then summarized the highlights of their concerns:

- For sweet lupin flour (GRN 000264), the test substance used in most of the safety studies cited in the notice were whole lupin seeds or lupin protein. There were three studies summarized in the notice on flour from individual lupin species. There was no study in the notice that used lupin flour derived from a mix of lupin species and varieties which is the subject of GRN 000264. In order for the studies to support the safe use of sweet lupin flour, the test article of the studies must be similar to the subject of the GRAS notice.
- The subchronic rat study (using *L. angustifolia* flour) had no concurrent controls and the data were compared with historical values from another laboratory. There were two human studies using *L. albus* flour. One study gave one (150 g) cookie/day enriched with lupin flour to subjects for 60 days and found no significant adverse effect. The second study, in men only, reported an average nitrogen digestibility level of 77% and an increase in plasma urea nitrogen.
- For GRN 000264, lupin is reported to be low in lysine, methionine, cysteine and calcium. George Weston Foods, Ltd. needs to state clearly the levels of these amino acids and calcium in the flour.
- For GRN 000264, *Lupinus albus* seed is reported to be very high in manganese and may be toxic. This was not addressed in the notice.
- For GRN 000262 and GRN 000263 phomopsin levels are not presented in the batch analyses presented in Appendix B-2. Moreover, the notifier did not provide the correct reference to the maximal established level of 5 ppb. The FSANZ document that the notifier refers to (http://www.foodstandards.gov.au/_srcfiles/TR1.pdf), states that in the absence of human data and in the absence of a NOEL in animals, it is not possible to derive a tolerable level for human exposure.
- Also for GRN 000262 and GRN 000263, more sensitive methods should be used to determine the levels of pesticide residues so that the established MRL can be met. Their levels may be <0.05 ppm as detected, but >0.02 ppm as established by FSANZ (Table B-3-1).
- For GRN 000263, subchronic toxicity data on lupin flour cannot support the safety of lupin hull fiber.
- For GRN 000262, the reviewer noted some minor issues. In Table 4 footnote b is missing. On P. 24 line 2, maybe 1.4 mg trypsin inhibitors/day? In Appendix B-4, Table B-3-1, why does the amount of insoluble fiber decrease during storage?

MEMORANDUM OF MEETING

Date: August 26, 2009

Time: 2:00 p.m. – 2:30 p.m.

Place: FDA, Center for Food Safety and Applied Nutrition, Office of Food Additive Safety, 4300 River Road, College Park, MD 20740, room 2073

Subject: GRN 000262, sweet lupin protein; GRN 000263 sweet lupin fiber; GRN 000264, sweet lupin flour

Participants:Visitor:

Melody Harwood

Cantox Health Sciences International

FDA:

Susan Carlson

HFS-255

Robert Martin

HFS-255

The meeting was held at the request of Ms. Harwood on behalf of Cantox's client, George Weston Foods, Ltd., for the purpose of discussing the steps needed to resubmit the previously withdrawn GRAS Notices for sweet lupin protein, sweet lupin fiber, and sweet lupin flour (Attachment). Ms. Harwood stated that George Weston Foods, Ltd. wished to pursue labeling as a condition of safe use for their lupin-based ingredients.

FDA personnel noted that because it has been established in the scientific literature that peanut-allergic individuals also react to lupin-based ingredients we believe it will be difficult to establish a basis for the general recognition of safety for lupin, even with a label statement.

FDA personnel and Ms. Harwood discussed the possibility of submitting food additive petitions instead of GRAS Notices. FDA personnel cautioned Ms. Harwood that given the well-known cross-reactivity of lupin proteins and peanut proteins and the lack of consumer awareness in the U.S. market, it would be difficult to establish the safe use of lupin ingredients, even with a regulation.

Ms. Harwood thanked FDA personnel for their time and stated that she would convey the information to George Weston Foods, Ltd.


Susan Carlson, Ph.D.

Attachment

(b) (5)



000165

Attachment

From: Melody Harwood
To: Carlson, Susan;
cc: Martin, Robert L;
Subject: RE: Request for Meeting on Lupin October 30, 2009
Date: Monday, August 24, 2009 3:45:45 PM

OK, sounds great. Thanks for your consideration. I look forward to seeing you on Wednesday.

Kind regards,
Melody

-----Original Message-----

From: Carlson, Susan [<mailto:Susan.Carlson@fda.hhs.gov>]
Sent: Monday, August 24, 2009 1:48 PM
To: Melody Harwood
Cc: Martin, Robert L
Subject: RE: Request for Meeting on Lupin October 30, 2009

Dear Melody,
Please plan on spending a few minutes (5 to 10 min) with Dr. Martin and myself following your meeting here Wednesday, August 26. We would like to discuss our current thinking on the allergenicity issues of lupin.
Thank you,
Susan

-----Original Message-----

From: Melody Harwood [<mailto:mharwood@cantox.com>]
Sent: Monday, August 24, 2009 12:01 PM
To: Carlson, Susan
Cc: Ashley Roberts
Subject: Request for Meeting on Lupin October 30, 2009

Hi Dr. Carlson,

I hope this finds you well and enjoying summer time in Maryland.

I am contacting you to request a meeting with our client, George Weston Foods, Australia, to discuss some of the issues that were identified by the agency during their review last fall of 3 GRAS Exemption Claims for their lupin ingredients. In particular, the current issue that our client is facing pertains to potential allergenicity of the lupin

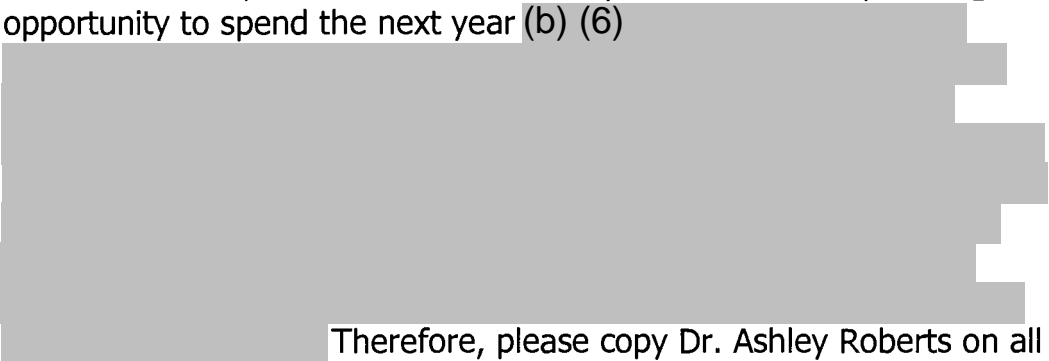
000166

proteins, as well as potential cross-reactivity of these proteins with other allergens (including peanut) and how to adequately label retail products containing these ingredients so as to properly inform any lupin- or peanut-sensitive individuals.

Our client has been working with several experts, including Dr. Steve Taylor, various clinicians, and members of Cantox, and would like to request a meeting with CFSAN to discuss this matter with all that need to be involved. It is requested that representatives from both OFAS and ONLDS be invited to attend this meeting, and I want to get your opinion if we should also invite a representative of the Office of Regulations, Policy and Social Sciences or of the Office of Compliance, as I believe this is an unprecedented issue? Perhaps even Michael Landa, Deputy Director for Regulatory Affairs, should be included? Our client will be coming from Australia and have already confirmed the availability of their other experts for October 30, 2009. I do realize that you usually need a couple of suggested days in order to find a date that suits the schedules of all involved, but I'm hoping that providing ample time before the meeting will allow for this date to be feasible to meet.

I look forward to your thoughts on this matter. I will be down in College Park on Wednesday, August 26th, and if there is an opportunity to discuss a meeting strategy with you and your colleagues I would be grateful and will gladly make myself available after 2 pm.

On another note, I am excited to inform you that I will be pursuing an opportunity to spend the next year (b) (6)



Therefore, please copy Dr. Ashley Roberts on all correspondence related to this meeting request, as he will continue to work with yourself and our client subsequent to my last day at Cantox to finalize a meeting date..

Please don't hesitate to let me know if you have any questions or concerns.

Kind regards,
Melody

Melody Harwood, M.Sc.
Associate Director, Business Development
Food and Nutrition
Cantox Health Sciences International

2233 Argentia Road, Suite 308
Mississauga, ON, L5N 2X7, CANADA
Tel: 905-542-2900, Extension 302
Fax: 905-542-1011
Mobile: 905-580-6693
mharwood@cantox.com

The 4th Practical Short Course on Functional Oils: Omega-3 Fatty Acids
Market Trends, Nutrition & Health, Utilization in Food Systems is August
24-25, 2009 in Chicago, IL! Ms. Lina Paulionis of Cantox will be
presenting, "Omega-3 Oils: Health Claims Global Perspectives" on August
24 at 10:00 a.m. Don't miss it!
<<http://home.scarlet.be/~tpm12374/smartshortcourses/pdf/4thFLipids.pdf>>
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000168

Carlson, Susan

SU

**From:** Kane, Rhonda R.**Sent:** Thursday, October 08, 2009 9:45 AM**To:** Billingslea, Felicia B; June, Geraldine A; Martin, Robert L; Carlson, Susan; Dinovi, Michael J; Luccioli, Stefano; Tarantino, Laura M**Subject:** RE: Outstanding Industry Issues About Lupine

To all,

[Redacted block containing a large area of text obscured by a grey box. On the right side of the redaction, the text "(b) (5)" is visible, indicating a FOIA exemption.

Rhonda R. Kane, MS, RD
Consumer Safety Officer (HFS-820)
Food Labeling and Standards Staff
ONLDS / CFSAN / FDA
CP 1, Room 4D-008
Phone: (301) 436-1803
Fax: (301) 436-2636
E-mail: Rhonda.Kane@fda.hhs.gov

From: Carlson, Susan**Sent:** Monday, October 05, 2009 3:09 PM**To:** Kane, Rhonda R.**Cc:** Billingslea, Felicia B; June, Geraldine A; Martin, Robert L**Subject:** RE: Outstanding Industry Issues About Lupin

Dear Rhonda,

(b) (5) [Redacted block containing a large area of text obscured by a grey box. The text "(b) (5)" is visible at the start of the redacted area, indicating a FOIA exemption.

Thank you Rhonda,
--Susan

From: Martin, Robert L**Sent:** Tuesday, September 29, 2009 7:42 AM**To:** Carlson, Susan**Cc:** Billingslea, Felicia B; June, Geraldine A; Kane, Rhonda R.**Subject:** RE: Outstanding Industry Issues About Lupin

000169

(b) (5)

Thanks.

Robert L. Martin

301-436-1219

From: Carlson, Susan

Sent: Monday, September 28, 2009 3:15 PM

To: Martin, Robert L

Cc: Billingslea, Felicia B; June, Geraldine A; Kane, Rhonda R.

Subject: FW: Outstanding Industry Issues About Lupin

Hello Bob,

(b) (5)

Thank you,
Susan

From: Kane, Rhonda R.

Sent: Monday, September 28, 2009 2:26 PM

To: Carlson, Susan

Subject: FW: Outstanding Industry Issues About Lupin

FYI

From: Dinovi, Michael J

Sent: Monday, September 28, 2009 12:54 PM

To: Kane, Rhonda R.

Subject: RE: Outstanding Industry Issues About Lupin

Rhonda

000170



(b) (5)

Mike

From: Kane, Rhonda R.
Sent: Monday, September 28, 2009 12:31 PM
To: Dinovi, Michael J
Subject: Outstanding Industry Issues About Lupin

Hi, Michael,

(b) (5)



Rhonda R. Kane, MS, RD
Consumer Safety Officer (HFS-820)
Food Labeling and Standards Staff
ONLDS / CFSAN / FDA
CP 1, Room 4D-008
Phone: (301) 436-1803
Fax: (301) 436-2636
E-mail: Rhonda.Kane@fda.hhs.gov

From: Billingslea, Felicia B
Sent: Monday, September 28, 2009 10:44 AM
To: 'Ashley Roberts'; Tarantino, Laura M
Cc: Ian Munro
Subject: RE: Meeting on Lupin

Hi Ashley,

I am aware of your requests and will ask one of my staff to follow up with you.

Thanks,
Felicia

From: Ashley Roberts [<mailto:aroberts@cantox.com>]
Sent: Wednesday, September 23, 2009 8:11 AM
To: Tarantino, Laura M
Cc: Ian Munro; Billingslea, Felicia B
Subject: RE: Meeting on Lupin

Dear Laura,

Many Thanks for your quick response and advice.

I will make contact with Felicia directly on this matter

Kind Regards

Ashley

000171

From: Tarantino, Laura M [mailto:Laura.Tarantino@fda.hhs.gov]
Sent: Wednesday, September 23, 2009 8:03 AM
To: Ashley Roberts
Cc: Ian Munro; Billingslea, Felicia B
Subject: RE: Meeting on Lupin

Hi, Ashley,

We had suggested that your initial contact be Felicia Billingslea in our labeling group, since the specific questions are primarily about labeling precedents. She or one of her staff will help arrange the meeting, and will keep Dr. Carlson and others in OFAS in the loop.

Laura Tarantino

From: Ashley Roberts [mailto:aroberts@cantox.com]
Sent: Tuesday, September 22, 2009 4:28 PM
To: Tarantino, Laura M
Cc: Ian Munro
Subject: FW: Meeting on Lupin

Dear Dr. Tarantino,

I just wanted to follow-up on some previous correspondence between Dr. Munro and yourself regarding the setting up of a meeting between the Agency, the manufacturer of lupin and Dr. Steve Taylor. Unfortunately, Dr. Munro has been out of the office recently and he has asked me to make contact with you again regarding this matter.

Dr. Munro has informed me that you recommended that we should try to set up a meeting by speaking firstly with one of your staff members whose name we are now seeking. I am led to believe that this person was not Dr. Carlson as outlined below. Please could you relay to me who this person was so that I might speak with them directly on this matter.

I look forward to receiving your response on this matter

Kind Regards

Ashley Roberts, PhD
VP, Food & Nutrition Group

CANTOX Health Sciences International
2233 Argentia Road, Suite 308
Mississauga, Ontario, Canada
L5N 2X7

T: 905-542-2900
F: 905-542-1011
E: aroberts@cantox.com
W: www.cantox.com

From: Ian Munro
Sent: Tuesday, July 14, 2009 2:17 PM
To: Tarantino, Laura M
Subject: RE: Meeting on Lupin

Hi Laura....Thanks for your email. No I am nor in Aspen this week but stuck working in Toronto. I have a hectic week this week but I would like to call you next week after Monday. So why don't I shoot you an email early next week and you can tell me a good time to call.....Thanks.....Ian

Ian C. Munro, Ph.D., F.A.T.S., FRCPath
Executive Vice President

000172

Senior Scientific Consultant
Cantox Health Sciences International
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Mississauga, ON L5N 2X7
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Fax: (905) 542-1011
imunro@cantox.com
www.cantox.com



Please consider the environment before printing this e-mail

From: Tarantino, Laura M [<mailto:Laura.Tarantino@fda.hhs.gov>]
Sent: Monday, July 13, 2009 4:10 PM
To: Ian Munro
Subject: RE: Meeting on Lupin

Hi, Ian,

Thank you so much for this background. It is very helpful. And I am sorry for my delay in responding, but I was on leave until last week, and I am just now catching up on email. Before I left, I did talk to a few folks, so I think we can work on setting up a meeting, and your notion of contacting Susan Carlson to coordinate is a good one. However, I'd be happy to discuss with you beforehand, if it will be helpful.

I am assuming you are in Aspen this week. I am not in College Park today, but should be there the rest of this week and most of next. I will plan to try to contact you early next week, or give me a holler before then if convenient.

Laura

From: Ian Munro [<mailto:imunro@cantox.com>]
Sent: Monday, June 29, 2009 10:26 AM
To: Tarantino, Laura M
Cc: Melody Harwood
Subject: RE: Meeting on Lupin

Laura,

Many thanks for your help so far. Here is a brief synopsis of what has happened to date.

We had a pre-submission meeting on September 10, 2007 with several OFAS people.

- Dr. Michael DiNovi, OFAS/DBGNR
- Dr. Ron Chanderbhan, OFAS/DBGNR
- Dr. Paulette Gaynor, OFAS/DBGNR
- Dr. Marcella Fruchter, OFDCER/DEC/COCB
- Dr. Jeremy Mihalov, OFAS/DBGNR

There didn't seem to be any concerns about the overall safety of lupin ingredients including lupin protein, flour and fibre until the potential allergenicity of lupin protein was mentioned, and even more so when the potential cross-reactivity with peanut was disclosed. Coming out of that meeting, it was recommended that we speak with a representative of the Office of Nutrition, Labelling and Dietary Supplements (ONLDS), so one of our people, Melody Harwood called and spoke with Rhonda Kane and explained the situation to her. As there currently isn't a policy for labelling for 'new' or cross-reactive allergens, she was not able to give clear guidance on what to do other than to comply with the ingredient labelling requirements, as per the regulations.

000173

We submitted the Notices for all three ingredients and yes, there were questions raised during the initial review phase (prior to withdrawal as a consequence of the allergen question), but none that were considered to be insurmountable. Our client indicated that the general feel they got in subsequent

discussions with OFAS was that the concerns of potential allergenicity and cross-reactivity were paramount and that the issue would not go away with simple conformity to the current ingredient and allergen labelling requirements, as these would not provide an avenue for warning or decreasing risk in lupin- or peanut-allergic individuals.

I believe that Dr. Susan Carlson handled the client's initial submission, and so we have planned to contact her to set up the meeting once we have settle on who should attend from your side

Thanks for your help.

Ian

From: Tarantino, Laura M [mailto:Laura.Tarantino@fda.hhs.gov]

Sent: Tuesday, June 16, 2009 3:22 PM

To: Ian Munro

Subject: RE: Meeting with CFSAN

Hello, Ian,

I did hear your voicemail. I have been, and am still today, away from my own office and travelling among buildings. I will give you a call back this week. I appreciate your wanting to address the cross-reactivity concern and willingness to work out something on labelling. Of course we will be happy to meet. We will have some discussion about who to have from our end. In the meantime, do let's reconnect later this week.

Best regards,

Laura

From: Ian Munro [mailto:imunro@cantox.com]

Sent: Tuesday, June 16, 2009 12:34 PM

To: Tarantino, Laura M

Subject: FW: Meeting with CFSAN

Dear Laura,

As per my voice mail of yesterday, I wanted to speak with you/get your feedback on the logistics of a meeting to discuss the labelling and self-affirmed GRAS status of our client's various lupin-derived ingredients.

These ingredients have been sa-GRAS, and the current issue that our client is facing pertains to potential allergenicity of the lupin proteins, as well as potential cross-reactivity of these proteins with peanut and how to adequately label retail products containing these ingredients so as to properly inform any lupin- or peanut-sensitive individuals. We have in the past discussed this concern with representatives of both the Office of Food Additive Safety and of the Office of Nutrition, Labeling and Dietary Supplements of CFSAN; however, this appears to be a situation without precedent under CFSAN policy and so a solution has not yet been reached.

Our client has been working with several experts, including Dr. Steve Taylor and various clinicians, as well as members of my staff, and would like to request a meeting with CFSAN to discuss this matter with all involved. It is anticipated that representatives from both OFAS and ONLDS would be invited to attend this meeting, and I want to get your opinion if we should also invite a representative of the Office of Regulations, Policy and Social Sciences or of the Office of Compliance. Perhaps even Michael Landa, Deputy Director for Regulatory Affairs, should be included?

I look forward to hearing from you at your earliest convenience. Many thanks in advance for your assistance with this matter.

Kind regards,

Ian

Ian C. Munro, Ph.D., F.A.T.S., FRCPATH
Executive Vice President
Senior Scientific Consultant
Cantox Health Sciences International
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Mississauga, ON L5N 2X7
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Fax: (905) 542-1011
imunro@cantox.com
www.cantox.com



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Are you attending NutrEvent in Lille, France, June 17-18, 2009?

Cantox's **Nigel Baldwin** will be speaking & participating in the Roundtable Discussion during the Regulatory Frameworks Seminar, June 17.

From: Bob McQuate
To: Fasano, Jeremiah;
cc: sanni@globepharma.com;
Subject: RE: GRN 295
Date: Tuesday, December 08, 2009 12:09:08 PM

Dear Dr. Fasano,

Since we are presently modifying GRN 295 to more tightly reflect common use in foods prior to 1958 as the basis for the GRAS determination, we will be removing safety studies such as the unpublished scientific data that you reference in our resubmission.

To the extent possible at this point, we would prefer to not make these unpublished data publicly available and would prefer to retain them as confidential. We did offer them as part of the subject notification with the understanding that their confidentiality could not be retained. Since we have withdrawn GRN 295, it is our preference that the subject unpublished studies be considered to be confidential.

We hope this is acceptable to you.

Sincerely,

Bob
Robert S. McQuate, Ph.D.
CEO & Co-Founder
GRAS Associates, LLC
20482 Jacklight Lane
Bend, OR 97702-3074
541-678-5522
mcquate@gras-associates.com
www.gras-associates.com

From: Fasano, Jeremiah [mailto:Jeremiah.Fasano@fda.hhs.gov]
Sent: Tuesday, December 08, 2009 8:46 AM
To: mcquate@gras-associates.com
Subject: 湏m需i敲

Dr. McQuate-

I wanted to confirm my assumption that Natreon does not consider any of the unpublished scientific data in the appendices to GRN 295 confidential. Please let me know if at your convenience if that is not the case.

On a tangential matter, I expect to mail out the official acknowledgement of withdrawal of GRN 295 tomorrow. Please let me know if you do not receive it in a reasonable amount of time.

Regards-

-Jeremiah Fasano

Jeremiah Fasano, Ph.D.
Consumer Safety Officer
Division of Biotechnology and GRAS Notice Review
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
Food and Drug Administration
Phone: 301-436-1173
Fax: 301-436-2964
Email: jeremiah.fasano@fda.hhs.gov

Mailing Address:

HFS-255
5100 Paint Branch Parkway
College Park, MD 20740

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August 3, 2009

Robert S. McQuate, Ph.D.
GRAS Associates, LLC
20482 Jacklight Lane
Bend, Oregon 97702-3074

Re: GRAS Notice No. GRN 000295

Dear Dr. McQuate

The Food and Drug Administration (FDA) has received the notice, dated June 29, 2009, that you submitted on behalf of Natreon, Inc. (Natreon) 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 July 2, 2009, filed it on July 10, 2009, and designated it as GRN No. 000295.

The subject of the notice is *Emblica officinalis* extract. The notice informs FDA of the view of Natreon that *Emblica officinalis* extract is GRAS, through common use in food, for use as an ingredient in nonalcoholic beverages and processed fruits and fruit juices at a level of 100 to 150 milligrams per serving.

In accordance with proposed 21 CFR 170.36(f), a copy of the information in this notice that conforms to the information described in the proposed GRAS exemption claim (proposed 21 CFR 170.36(c)(1)) is available for public review and copying via the FDA home page at <http://www.fda.gov>. To view or obtain an electronic copy of this information, follow the hyperlinks from the "Food" topic to the "Food Ingredients and Packaging" section to the "Generally Recognized as Safe (GRAS)" page where the GRAS Inventory is listed. If you have any questions about the notice, contact me at 301-436-1173.

Sincerely yours

Jeremiah Fasano
Division of Biotechnology and
GRAS Notice Review
Center for Food Safety
and Applied Nutrition

DEPARTMENT OF HEALTH AND HUMAN SERVICES

August 3, 2009

Robert S. McQuate, Ph.D.
GRAS Associates, LLC
20482 Jacklight Lane
Bend, Oregon 97702-3074

Re: GRAS Notice No. GRN 000295


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Sincerely yours,


Jeremiah Fasano
Division of Biotechnology and
GRAS Notice Review
Center for Food Safety
and Applied Nutrition

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DEPARTMENT OF HEALTH AND HUMAN SERVICES

**FOOD AND DRUG ADMINISTRATION
MEMORANDUM OF TELECONFERENCE**

DATE: November 16, 2009

TIME: 5:00 PM

PHONE NUMBER: 541-728-1492

PARTICIPANTS:

FDA

Jeremiah Fasano

HFS-255

External

Robert McQuate

GRAS Associates, LLC

SUBJECT: Status of GRN 295 (*Emblica officinalis* extract)

I contacted Robert McQuate by phone to discuss the status of GRN 295. Dr. McQuate is the agent for Natreon, Inc. (Natreon), the notifier. Dr. McQuate then returned my call.

I explained to Dr. McQuate that our preliminary review of GRN 295 identified a significant structural problem with the notice. Even though the basis of the GRAS notice was common use in food prior to 1958, it contained large quantities of extraneous material about the benefits of various intended uses of the ingredient, copious references to what appeared to be medicinal uses, and also presented scientific studies purporting to demonstrate the safety of various intended uses of *Emblica officinalis* extract. In addition, some of the references were problematic in terms of reliability or relevance.

OFAS considered that the notice was not sufficiently focused and clear to make an adequate case for the GRAS status of the intended use in food. I asked Dr. McQuate to consider advising Natreon to withdraw the notice (without prejudice to future submissions) and resubmit a new notice that strictly focused on well-documented evidence of widespread use of the ingredient in *food* prior to 1958. I also suggested that if Natreon pursued this course, the firm should limit discussion of scientific studies to those recent data or other information (if any) that might be considered to weaken a GRAS determination based on common use in food (i.e., evidence of toxicity or potentially adverse physiological effects), and why such data and information did not do so in this case.


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Dr.. McQuate requested an opportunity to review the notice and speak to me again about this matter. We arranged another discussion for the afternoon of Thursday, November 19th.


Jeremiah Fasano

(b) (5)



From: [Fasano, Jeremiah](#)
To: ["mcquate_gras-associates.com";](#)
Subject: FR Notices Discussing Common Use in Food Outside the US
Date: Thursday, November 19, 2009 2:00:25 PM
Attachments: [50FR27294_1986-07-02.pdf](#)
[53FR16544_1988-05-10.pdf](#)

Dr. McQuate-

Per our discussion today, I'm passing on two FR notices discussing the agency's thinking on how to assess evidence of common use in food prior to 1958 where that evidence is based on food use outside the United States. I hope you will find them useful.

I look forward to hearing from you.

Regards-

-Jeremiah Fasano

Jeremiah Fasano, Ph.D.
Consumer Safety Officer
Division of Biotechnology and GRAS Notice Review
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
Food and Drug Administration
Phone: 301-436-1173
Fax: 301-436-2964
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(i) Extension of loans beyond the original repayment terms;

(ii) Deterioration in the borrower's affairs sufficient to cause the lending institution to look to the sale of collateral for repayment;

(iii) Loans to unprofitable or undercapitalized business;

(iv) Special problems arising from conditions of a given industry.

(b) *Doubtful*. (1) Loans classified "Doubtful" would exhibit discernible loss potential where some, but not complete loss, seems very likely but there is still sufficient uncertainty that permits the asset to remain on the books (at its full value). In addition, a Doubtful loan could reflect the fact that the primary source of repayment is gone and doubt exists as to the quality of the secondary source of repayment.

(2) Doubtful classification, would most likely not be repeated at a subsequent examination because there should be enough time to resolve pending factors. If pending events did not occur and repayment was deferred awaiting new developments, a Loss classification normally would be warranted.

(3) *Loss*. A loan classified as "Loss" is considered uncollectible and of such little value that continuance as an asset is not warranted. A loss classification does not mean that a loan doesn't have recovery or salvage value, but simply that it is not practical or desirable to defer writing off all (or a portion) of a basically worthless asset, even though partial recovery may be effected in the future.

By the Federal Home Loan Bank Board.
Jeff Sconyers,
Secretary.

[FR Doc. 85-15838 Filed 7-1-85; 8:45 am]

BILLING CODE 6720-01-M

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration

21 CFR Part 170

[Docket No. 84N-0080]

Eligibility for Classification of Food Substances as Generally Recognized as Safe

AGENCY: Food and Drug Administration.

ACTION: Proposed rule.

SUMMARY: The Food and Drug Administration (FDA) is proposing to amend its regulations to recognize that a substance that was used in food prior to 1958 can be shown to be generally recognized as safe (GRAS) through experience based on its common use in

food outside, as well as in, the United States. FDA is proposing this amendment in response to a recent court decision that declared invalid an agency regulation that had restricted the experience that could provide the basis for general recognition of safety to experience in the United States. FDA is also proposing to delineate the showing that must be made to establish that a substance is GRAS on the basis of its foreign use.

DATE: Written comments by September 3, 1985.

ADDRESS: Written comments to the Dockets Management Branch (HFA-305), Food and Drug Administration, Rm. 4-62, 5600 Fishers Lane, Rockville, MD 20857.

FOR FURTHER INFORMATION CONTACT: Gerard L. McCowan, Center for Food Safety and Applied Nutrition (HFF-335), Food and Drug Administration, 200 C St. SW., Washington, DC 20204, 202-472-5878.

SUPPLEMENTARY INFORMATION:

I. Background

The Federal Food, Drug, and Cosmetic Act (the act) states that a substance that may become a component or otherwise affect the characteristics of a food is a "food additive" unless it is GRAS (21 U.S.C. 321(s)). Section 201(s) of the act also states that the term "food additive" does not include:

- (1) A pesticide chemical in or on a raw agricultural commodity; or
- (2) A pesticide chemical to the extent that it is intended for use or is used in the production, storage, or transportation of any raw agricultural commodity; or
- (3) A color additive; or
- (4) Any substance used in accordance with a sanction or approval granted prior to the enactment of this paragraph pursuant to this Act, the Poultry Products Inspection Act (21 U.S.C. 451 and the following) or the Meat Inspection Act or March 4, 1907 (34 Stat. 1260), as amended and extended (21 U.S.C. 71 and the following); or
- (5) A new animal drug.

21 U.S.C. 321(s)(1)-(5).

According to the act, a substance is GRAS if it is generally recognized, among experts qualified by scientific training and experience to evaluate its safety, as having been adequately shown through scientific procedures, or in the case of a substance used in food prior to January 1, 1958, through either scientific procedures or experience based on common use in food, to be safe under the conditions of its intended use.

In 1974, FDA proposed to adopt definitions for the terms "common use in

food" and "scientific procedures" (39 FR 34194, 34195; September 23, 1974). Only the former term is relevant to the present rulemaking.

The agency proposed to define "common use in food" to mean "a substantial history of consumption of a substance by a significant number of consumers in the United States." In the preamble to that proposal, the agency stated that it did not believe that use in a foreign country would support a GRAS determination based upon experience from common use. FDA explained that reported use in foreign countries often cannot be verified, and that the experience based on such use cannot be monitored or evaluated. The agency also stated that food consumption patterns and differences between cultures make it impossible to assess whether a history of use abroad would be comparable to a history of use in the United States (39 FR 34195). The agency did not receive any comments on this definition, and as a result, on December 7, 1976 (41 FR 53600), FDA adopted the definition without change. This definition ultimately was codified at 21 CFR 170.3(f).

On September 15, 1983, however, the United States Court of Appeals for the Ninth Circuit declared § 170.3(f) to be invalid. *Fmali Herb, Inc. v. Heckler*, 715 F.2d 1385 (9th Cir. 1983). The court ruled that by restricting "common use in food" to mean use only in the United States, FDA had imposed a restriction that did not comport with either the literal terms of the act or with the purpose of the common use in food exception, as articulated by the legislators (715 F.2d at 1390). Nevertheless, in striking down the regulation the court stated:

Under section 201(s), the important inquiry is whether a substance added to food is "generally recognized among experts qualified by scientific training and experience to evaluate its safety, as having been adequately shown * * * to be safe under the conditions of its intended use." Experience based on common use in food serves only as a means of showing safety. If the foreign experience cited by the proponent does not clearly demonstrate safety, because of doubts about cultural comparability or the adequacy of public health data, then the substance must be deemed a food additive. As the overriding purpose of the Food, Drug, and Cosmetic Act is to protect the public health, the burden of proof of safety to be borne by a proponent of an ingredient is heavy * * *. In practice, evidence of foreign use of an ingredient, standing alone, may rarely or never be enough to establish safety. An FDA regulation establishing a threshold of evidence needed to constitute sufficient proof of safety based on common use would be entitled to substantial judicial deference. * * *

715 F.2d at 1390-1391 (citations omitted).

II. Proposed Regulations

In response to the Ninth Circuit's decision, FDA has reevaluated its concerns about basing a conclusion that a substance that was in use before 1958 is safe solely on a history of common use outside the United States. The agency has attempted to determine whether there are circumstances in which these concerns could be allayed and thus in which a history of use outside the United States could be relied upon to establish the safety of such a substance. The following summarizes the agency's concerns and its tentative conclusions about those concerns.

A. Availability and Verifiability of Information About the History of Use of a Substance in Food

For a substance to be GRAS on the basis of a history of common use in food, there must be consensus among the community of qualified experts that the use of the substance is safe. For such a consensus to be possible, information about the use of the substance must be generally available. General availability is the result of documentation of the information, usually by publication.

The importance that FDA attaches to the availability of information about the safety of a substance in establishing that substance's GRAS status is evidenced by the fact that the agency incorporated a requirement of general availability into its current regulations (§ 170.30(c)).

In addition to being generally available, information on the history of use of a substance must be verifiable. To provide adequate evidence that a substance has been widely used, FDA believes that an independent source that confirms the history of the use of the ingredient must be available.

For substances with a history of use in food in the United States that pre-dates 1958, there usually is information available, much of it published, that documents the use of the substance in food. Moreover, FDA usually can verify quite readily the facts relating to the use of the ingredient. It can request that users of the substance supply use information, or it can utilize its network of regional offices to obtain such information.

It is more difficult to obtain information about, and to verify, the use of a substance outside the United States. Published information on the substance may not be available in the United States, or it may be written in a language other than English. Foreign language articles would have to be translated, and the translations authenticated. Distance and language

barriers also make it difficult for FDA to locate sources outside the United States that could provide information about the substance and that could verify claims about its use.

Therefore, FDA has tentatively concluded that it is necessary to require that a claim that a substance is GRAS based on its common use in food outside the United States before 1958 be documented and be verified by evidence that corroborates the use of the substance. Such a claim must also demonstrate that the information that supports the GRAS status of the ingredient is generally available. FDA would consider the information to be "generally available" if it is widely available in the country in which use has occurred and readily available to interested qualified experts in this country. Finally, the information must demonstrate that the substance has in fact been used as a food ingredient and not as a drug, tonic, or folk remedy.

B. Adequacy of Information About the History and Circumstances of Use of a Substance

For experts qualified by scientific training and experience to be able to agree that a substance has been shown to be safe under the conditions of its intended use, they must be presented with evidence that is adequate to allow them to make such a judgment about the ingredient. For them to make a judgment on the basis of evidence of experience based on common use in food, that evidence must include, although not necessarily be limited to, the following:

1. Data on the identity of the substance and information to show how the substance is distinguished from similar substances.
2. Information on the production, storage, and handling of the substance and on the consequences if the substance is not handled properly.
3. Information on the use of the ingredient, such as its technical effect in food, the amount added, and the foods to which it is added.
4. Information on the cultural context of the use of the substance, such as who eats the substance, how often, on which occasions, with what other foods, or in lieu of what other foods.
5. Information on the dietary habits in the country where use of the substance occurs.
6. Information on the public health practices in the country where the use of the substance occurs. This includes information about the means, if any, by which the country records any public health problems associated with a food or food ingredient.

7. Information on reports of the consequences of consuming the substance abusively.

8. Information on any health problems associated with use of the ingredient in the country where use of the ingredient occurs.

FDA believes that these data represent the minimum amount of information necessary to permit an expert to determine (1) whether a substance has been in common use, and if so, (2) whether the experience with the ingredient in common use establishes that use of the ingredient is safe. These kinds of data are ordinarily generally available for substances having a history of common use in food in the United States. Much of this information is often totally lacking, however, for substances used in food outside the United States. Nevertheless, this information must be available to support a claim that a substance is GRAS on the basis of such use.

C. Determination of Safety Based on Information About the History and Circumstances of Use of a Substance

General recognition of safety requires not only the general availability of appropriate evidence on the substance but also general agreement on the interpretation of the evidence. FDA believes that this general agreement can occur only when similarly qualified experts share an understanding of the concept of safety.

"Safety" is a conclusion based on evidence, but it is also a subjective and relative term. Although evidence used in a safety decision is concrete and includes such items as documentation of the use and of the experience of using the substance in food, the interpretation of the evidence reflects the training, experience, and cultural values of the person making the decision. These factors affect the decisions about the importance or weight assigned to various reports on the substance, judgments about the probability and seriousness of adverse effects resulting from exposure to the substance, and determinations about whether there are enough data to support a decision.

It is entirely possible for two groups of experts with similar training and experience in the evaluation of the safety of a food substance but who live in different cultures to disagree on a safety decision based upon experience through common use in food. Each group would be biased by the values held by its own culture.

Each society collectively decides to accept certain risks associated with consuming a particular food because it

decides that the benefits from consuming that food outweigh the risks. Among the factors that influence a society's selection of foods are the abundance (or conversely, the scarcity) of the food; religious taboos; the social significance, such as social rank, of consumption of a particular food; ignorance of nutritional principles; and religious or social ritual (resulting in the consumption of such foods as wine or tea). Thus, for example, a society may tolerate a food that has a known hazard associated with its consumption because the society has no economically feasible alternative.

Consequently, any decision made in another country that an ingredient whose use pre-dates 1958 is safe based upon experience through common use in food must be reviewed by the community of experts that historically has made safety assessments in this country and whose views reflect the values of this society. The need for review by this community of experts is quite independent of the quantity or quality of data supporting the decision or of the qualifications of the experts who rendered the original decision. The review needs to be performed to assure that the substance has been shown to be safe, as that term is understood in this country.

As a practical matter, general recognition among the community of experts in the United States that a substance is safe is not likely to be achieved unless information on that substance is distributed widely in the United States and thus is generally available to members of the community. The opinions of this diverse community would have to be obtained and examined to determine whether there is in fact consensus on the safety of the substance. Because information about the use of a substance may be generally available without being widely circulated in this country and because of the difficulties in demonstrating consensus, the appropriate alternative would be for the agency itself to judge whether a substance has been shown to be GRAS based upon its history of common use in food outside the United States. The experts at FDA are selected from the community of experts who are qualified to evaluate the safety of food ingredients, and, therefore, the opinions of FDA are representative of those held by the larger community. Moreover, FDA has been making determinations about the GRAS status of food ingredients since the passage of the Food Additives Amendment in 1958.

In making determinations about the GRAS status of food ingredients, FDA

takes into account not only the advanced state of scientific expertise but also the high standard of living and degree of public health that have been attained in the United States. FDA has established a number of programs to ensure that the safety of the American food supply continues to meet these high standards. Among these programs are the petition procedures for GRAS affirmation, the comprehensive review of GRAS ingredients, and the adoption of safety criteria and guidelines for toxicological studies ("Toxicological Principles for the Safety Assessment of Direct Food Additives and Color Additives Used in Food," 1982). Through these actions, FDA has established a standard of rigor that must be met in an effort to demonstrate that a food or a food ingredient is safe. As a consequence, any substance whose safety has not been demonstrated by a showing of appropriate rigor may be considered by FDA not to have been shown to be safe. The showing that FDA is proposing to require in § 170.30(c)(2) applies this standard of rigor to GRAS claims that are based on common use of an ingredient outside the United States before 1958.

Moreover, FDA also is proposing to state in § 170.30(c)(2) that a person who claims that an ingredient in use before 1958 is GRAS on the basis of its common use outside the United States should petition the agency for concurrence in the ingredient's GRAS status. Although persons normally are free to make their own determination about whether a substance is GRAS, the agency believes that there are two significant reasons why they should refrain from making such a determination for a substance with a history of use exclusively or primarily outside the United States until they have obtained FDA's concurrence that the ingredient is GRAS:

First, because information on the history and circumstances of use of a substance outside the United States is usually not widely circulated in this country, it cannot ordinarily be assumed that the community of experts in the United States generally recognizes that the safety of that substance has been established based upon its history of use abroad. Thus, it is necessary to obtain FDA's opinion as to whether general recognition of the safety of a substance exists among that community. If one introduces such a substance into interstate commerce without obtaining FDA's concurrence, one takes the risk that the substance will be found not to be GRAS and thus to be an illegal food additive.

Second, also because of the lack of circulation of information, FDA may not have any knowledge about many substances with a history of use outside this country. Consequently, in the absence of information about a substance, the agency may seize the substance or food containing the substance or detain these items when they are brought into the United States. Although it may ultimately be shown that the substance is GRAS, the seizure or detention of the substance will delay its use. Such a delay can be avoided if interested persons petition the agency before introducing the substance into interstate commerce.

In accordance with this discussion and the agency's tentative conclusions, FDA is proposing to revise its procedural regulations to establish that a substance in use before 1958 may in fact be eligible for GRAS status based upon its history and circumstances of use in food outside of the United States. The agency is revising the definition of "common use in food" in § 170.3(f) so that it no longer stipulates that consumption of the substance must have occurred only in the United States. The agency is proposing to add a new paragraph (c)(2) to § 170.30 that specifies the information that is required to establish that a substance is GRAS based upon a history of common use in food when that use has occurred exclusively or primarily outside of the United States. Proposed § 170.30(c)(2) requires verification of the history of common use in food and sufficient information about the use to determine the context in which the use occurred and to establish that use of the substance is safe within the meaning of the act. The proposed regulation also suggests that persons claiming GRAS status for an ingredient on the basis of its common use in food outside the United States obtain FDA concurrence that the substance is GRAS.

FDA is also proposing to revise the procedures for GRAS affirmation petitions in § 170.35 to make clear that a request for GRAS affirmation may be based upon either scientific procedures or a history of common use in food. The regulation currently stipulates that the petition be based upon scientific procedures.

The agency has determined under 21 CFR 25.24(b)(12) (April 28, 1985; 50 FR 16636) that this action is of a type that does not individually or cumulatively have a significant effect on the human environment. Therefore, neither an environmental assessment nor an environmental impact statement is required.

FDA, in accordance with the Regulatory Flexibility Act, has considered the effect that this proposal would have on small entities including small businesses. FDA certifies in accordance with section 605(b) of the Regulatory Flexibility Act that no significant economic impact on a substantial number of small entities will derive from this action.

In accordance with Executive Order 12291, FDA has carefully analyzed the economic effects of this proposal and has determined that the final rule, if promulgated, will not be a major rule as defined by the Order.

The agency's findings of no major economic impact and no significant impact on a substantial number of small entities, and the evidence supporting these findings, are contained in a threshold assessment which may be seen in the Dockets Management Branch (address above).

List of Subjects in 21 CFR Part 170

Administrative practice and procedure, Definitions, Food additives, Food additive safety.

Therefore, under the Federal Food, Drug, and Cosmetic Act, it is proposed that Part 170 be amended as follows:

PART 170—FOOD ADDITIVES

1. The authority citation for 21 CFR Part 170 is revised to read as follows:

Authority: Secs. 201(s), 402, 409, 701(a), 52 Stat. 1048-1047 as amended, 1055, 72 Stat. 1784-1788 as amended (21 U.S.C. 321(s), 342, 348, 371(a)); 21 CFR 5.11.

2. In § 170.3 by revising paragraph (f), to read as follows:

§ 170.3 Definitions.

(f) "Common use in food" means a substantial history of consumption of a substance solely for food use by a significant number of consumers.

3. In § 170.30 by redesignating paragraph (c) as paragraph (c)(1) and by adding new paragraph (c)(2), to read as follows:

§ 170.30 Eligibility for classification as generally recognized as safe (GRAS).

(c) * * *

(2) A substance used in food prior to January 1, 1958, may be generally recognized as safe through experience based on its common use in food when that use occurred exclusively or primarily outside of the United States if the information about the experience establishes that the use of the substance is safe within the meaning of the Federal

Food, Drug, and Cosmetic Act. Common use in food prior to January 1, 1985, that occurred outside the United States shall be documented by published or other information and shall be corroborated by information from a second, independent source that confirms the history and circumstances of use of the substance. The information used to document and to corroborate the history and circumstances of use of the substance must be generally available; that is, it must be widely available in the country in which the history of use has occurred and readily available to interested qualified experts in this country. Persons claiming GRAS status for a substance based on its common use in food outside of the United States should obtain FDA concurrence that the use of the substance is GRAS.

4. In § 170.35 by revising the introductory text of paragraph (c)(1), to read as follows:

§ 170.35 Affirmation of generally recognized as safe (GRAS) status.

(c)(1) Persons seeking the affirmation of GRAS status of substances as provided in § 170.30(e), except those subject to the NAS/NRC GRAS list survey (36 FR 20546), shall submit a petition for GRAS affirmation pursuant to Part 10 of this chapter. Such petition shall contain information to establish that the GRAS criteria as set forth in § 170.30(b) or (c) have been met, in the following form:

Interested persons may, on or before September 3, 1985, submit to the Dockets Management Branch (address above) written comments regarding this proposal. Two copies of any comments are to be submitted, except that individuals may submit one copy. Comments are to be identified with the docket number found in brackets in the heading of this document. Received comments may be seen in the office above between 9 a.m. and 4 p.m., Monday through Friday.

Dated: May 6, 1985.

Frank E. Young,
Commissioner of Food and Drugs.

Margaret M. Heckler,
Secretary of Health and Human Services.

[FR Doc. 85-15787 Filed 7-1-85; 8:45 am]
BILLING CODE 4160-01-M

DEPARTMENT OF THE TREASURY

Internal Revenue Service

26 CFR Part 1

[LR-31-85]

Tax-Exempt Entity Leasing; Proposed Rulemaking

AGENCY: Internal Revenue Service, Treasury.

ACTION: Notice of proposed rulemaking by cross reference to temporary regulations.

SUMMARY: This document provides proposed regulations regarding tax-exempt entity leasing. Changes to the applicable tax law were made by the Tax Reform Act of 1984. In the Rules and Regulations portion of this Federal Register, the Internal Revenue Service is issuing temporary regulations relating to tax-exempt entity leasing; the text of those temporary regulations also serves as the comment document for this notice of proposed rulemaking.

DATES: Written comments and requests for a public hearing must be delivered or mailed by September 3, 1985. In general, the regulations are proposed to apply to leases involving tax exempt entities entered into after May 23, 1983, and to property placed in service by the taxpayer after May 23, 1983. The revisions to safe-harbor lease reporting requirements are proposed to apply to such lease agreements executed after June 30, 1985.

ADDRESS: Please mail or deliver comments to: Commissioner of Internal Revenue, Attention: CC:LR:T (LR-31-85), 1111 Constitution Avenue, N.W., Washington, D.C. 20224.

FOR FURTHER INFORMATION CONTACT: Robert Beatson of the Legislation and Regulations Division, Office of Chief Counsel, Internal Revenue Service, 1111 Constitution Avenue, N.W., Washington, D.C. 20224 (Attention: CC:LR:T) (202-566-3590).

SUPPLEMENTARY INFORMATION:

Background

The temporary regulations in the Rules and Regulations portion of this issue of the Federal Register add new §§ 1.48-12T, 1.168(f)(8)-1T, and 1.168(j)-1T to Part 1 of Title 26 of the Code of Federal Regulations. When § 1.48-12T is promulgated as a final regulation, it would be renumbered as § 1.48-12 of Part 1 of Title 26 of the Code of Federal Regulations. When § 1.168(f)(8)-1T is promulgated as a final regulation, it would be renumbered as § 1.168(f)(8)-1

there is no sharp distinction between the use of substance as a food and as a "folk remedy." It suggested that the definition should be: "Common use in food means a substantial history of consumption of a substance for food or other use by a significant number of consumers." Another comment agreed with this view and stated that "since FDA's concern is whether substances are safe, all safety data from whatever source should be examined."

The agency has reviewed these comments and believes that the respondents misinterpreted the agency's intent in including the word "solely" in the proposed definition of "common use in food." The agency does not intend to exclude from GRAS eligibility food substances that also have nonfood uses. However, GRAS determination based upon common use in food depends, by definition, upon food use. The agency used the words "solely for food use" to emphasize this important point. Sufficient data must exist that document the safe use of the substance in food. Data on the use of a substance as a drug, for example, cannot substitute for data on food use.

However, the agency recognizes that inclusion of the word "solely" in § 170.3(f) may cause confusion. Therefore, the agency has removed that word from § 170.3(f).

The agency still considers it necessary to emphasize the concept that eligibility for GRAS status through experience based on common use in food prior to January 1, 1958, must be based solely on use of the substance in food. Consequently, the agency has included language to this effect in new § 170.30(c)(1). Because this revision merely clarifies the agency's intent expressed in the proposal, further opportunity for comment is not necessary.

2. One comment requested modifications in the safety standards for food and food ingredients.

The agency advises that this issue is outside the scope of this rulemaking. The agency further advises that it has no authority to modify the safety standards that are prescribed by the act.

3. Six comments objected to the last sentence of proposed § 170.30(c)(2), which reads: "Persons claiming GRAS status for a substance based on its common use in food outside of the United States should obtain FDA concurrence that the use of the substance is GRAS." Five comments asserted that the requirement for FDA concurrence imposes more stringent requirements upon foods that are GRAS based upon foreign experience than upon those claimed to be GRAS based

upon U.S. experience. Specifically, the comments asserted that: (a) Such stringent restrictions are not required by statute and exceed FDA authority; (b) products that are not food additives within the meaning of the act are exempt from premarket approval; (c) the requirement is not in agreement with the act or with the ruling of the court in *Fmali Hert, Inc. v. Heckler*, 715 F.2d 1385 (9th Cir. 1983); and (d) FDA should reconsider its reasons for justifying the need for concurrence.

FDA has considered these comments and acknowledges that persons have the right to make independent GRAS determinations on food substances. Indeed, the preamble to the proposal stated that "persons normally are free to make their own determination about whether a substance is GRAS."

However, FDA is charged with the responsibility of protecting interstate commerce from adulterated foods. When a food substance is offered for import, FDA must judge whether that substance is adulterated on the basis of the information known about it. If, in advance of offering the substance for import, the importer has petitioned FDA and obtained agency concurrence that the substance is GRAS, the substance will enter the United States with little or no problem.

On the other hand, if the importer has failed to seek FDA concurrence that the use of the substance is GRAS, and if the substance has no history of use in the United States, the agency cannot simply assume that the substance is GRAS. Therefore, it has little choice but to find that the substance appears to be adulterated. Under section 801(a) of the act (21 U.S.C. 381(a)), FDA is authorized to detain articles that appear to be adulterated and to refuse admission to those articles.

A person whose product has been detained has a right to request a hearing to review the initial determination that the substance appears to be adulterated, and FDA will listen to relevant evidence presented at such a hearing. While the agency will consider the evidence on this question at the hearing, determinations on GRAS status are not usually the type of simple and straightforward decisions that are appropriately made in the context of a detention hearing. Therefore, it seems likely that such hearings will rarely result in a finding that a substance is GRAS.

Furthermore, detention hearings are high pressure situations in which a decision must be made as quickly as possible because the goods are either waiting on the docks or being held under bond. Thus, detention hearings are ill-

suited to consideration of whether the use of a food ingredient is GRAS based on its history of use outside the United States.

Evidence on the effects of the use of a substance usually must be evaluated by FDA scientists trained in such disciplines as toxicology, chemistry, and epidemiology before the agency can make a determination as to whether the history of use of the substance provides an assurance of safety. Thus, except in rare cases in which the evidence of pre-1958 use of a substance provides overwhelming evidence of its safety (e.g., when there was specific approval granted for its use before 1958 by a foreign government), questions about whether use of the substance is GRAS are likely to remain unresolved at the detention hearing. If such questions persist, the hearing officer will likely affirm the finding that the substance appears to be an unapproved, and therefore unsafe, food additive.

For these reasons, prudence suggests that an importer who has made an independent determination that a substance is GRAS on the basis of its history of use outside the United States seek FDA concurrence in that judgment, by means of a GRAS affirmation petition, before seeking to bring the product into this country. The last sentence in § 170.30(c)(2) incorporates this suggestion into FDA's regulations. It does not require that such concurrence be obtained, establish a premarket approval requirement, or establish more stringent requirements for foods that allegedly are GRAS based on foreign experience than for those that allegedly are GRAS based on experience in the United States. It merely reflects the fact that, to protect the safety of the food supply, section 801(a) of the act requires that FDA deny entry into this country to foods that even appear to be adulterated, and that it is to the advantage of the importer to have questions about possible adulteration resolved before the food is offered for entry.

Finally, the last sentence in § 170.30(c)(2) is fully consistent with *Fmali Herb, Inc. v. Heckler*. That decision states that FDA cannot refuse to consider evidence of safety based on use of a substance outside the United States before 1958. The last sentence in § 170.30(c)(2) does not purport to do so. The agency is fully prepared to consider such evidence. The sentence in question makes clear, however, that the agency prefers to be given such evidence before a product is offered for import and in a context other than a detention hearing.

For the foregoing reasons, FDA has not made any changes in the last sentence in § 170.30(c)(2) in response to the comments.

4. One comment contended that FDA should not deny an exemption from premarket approval simply because information supporting the safety of a substance is not readily available in this country, and that FDA's ability to obtain data is no reflection upon the validity of data.

Given its long history of regulating the food supply in the United States, FDA generally has some information relating to the safety of substances used in food in the United States. The same is not necessarily true for substances used in foreign countries. FDA's ability to obtain comparable information on the use of substances in foreign countries may be no reflection on the validity of the information, but it does affect the agency's ability, and the ability of qualified experts in this country, to assess the safety of the food substance.

5. One comment noted that in deciding whether to allow imports into the United States the agency is not entitled to create legal requirements (i.e., the imports are on an FDA approved substances list) because it is convenient to have a list of approved substances.

It is to the advantage of an importer to have the substances of its product on an FDA-approved list, which would occur if FDA concurred in the GRAS determination. This listing would eliminate delays and uncertainties regarding the food product's entry into the United States. However, FDA has not created any legal requirements to this effect.

6. Three comments stated that a requirement for FDA concurrence would have a catastrophic economic impact upon importers.

The agency does not agree with the assertion that a requirement for FDA concurrence in a foreign determination of GRAS would have a catastrophic economic impact upon importers. This rule serves to open U.S. markets to products not previously sold in this country. Any expense associated with the submission of a petition seeking FDA concurrence should be minimal because an individual or firm making an independent GRAS determination should already have the information needed to support a general recognition of safety.

Due to these considerations, the agency has not modified its regulation in response to these comments.

7. One comment was concerned that FDA might exclude data on foreign usage after January 1, 1958, in making GRAS determinations.

The agency emphasizes that it regulates substances used outside of the United States after 1958 in the same manner as those used in the United States after 1958. The act allows substances that were used in food prior to January 1, 1958, to be GRAS either through experience based on common use in food or through scientific procedures (21 U.S.C. 321(s) and 21 CFR 170.30(a)). All substances first used in food after this date can be GRAS only on the basis of scientific procedures (21 CFR 170.30(b)).

8. Two comments recommended that the GRAS provisions on animal food substances also be revised to be consistent with the *Fmali* court decision.

The agency agrees that the court decision affects the GRAS provisions on animal food substances and has decided to initiate a rulemaking for the GRAS provisions on animal food substances. A notice of proposed rulemaking will be published in a future issue of the Federal Register.

III. Environmental Impact

The agency has previously considered the environmental effects of this rule as announced in the proposed rule (July 2, 1985; 50 FR 27294 at 27296). No new information or comments have been received that would affect the agency's previous determination that there is no significant impact on the human environment and that an environmental impact statement is not required.

IV. Economic Impact

In accordance with Executive Order 12291 and the Regulatory Flexibility Act, the agency previously considered the potential economic effects of this rule including its potential effect on small entities, including small businesses. In accordance with Executive Order 12291 and section 605(b) of the Regulatory Flexibility Act, the agency has determined that this rule would not be a major rule, and that no significant impact on a substantial number of small entities would derive from this action. As previously mentioned, the agency received three comments that claimed that this rule would have adverse economic effects on businesses. However, the agency did not receive any new information upon which to base a reconsideration of its previous determination.

The agency's findings of no major economic impact and no significant impact on a substantial number of small entities, and the evidence supporting these findings, are contained in a threshold assessment that was prepared in conjunction with the notice of proposed rulemaking and which may be

seen in the Dockets Management Branch (HFA-305), Food and Drug Administration, Rm. 4-62, 5600 Fishers Lane, Rockville, MD 20857.

V. Paperwork Reduction Requirements

Section 170.35(c)(i) of this final rule contain information collection requirements that were submitted for review and approval to the Director of the Office of Management and Budget (OMB), as required by section 3507 of the Paperwork Reduction Act of 1980. The requirements were approved and assigned OMB control number 0910-0132.

List of Subjects in 21 CFR Part 170

Administrative practice and procedure, Food additives.

Therefore, under the Federal Food, Drug, and Cosmetic Act and under authority delegated to the Commissioner of Food and Drugs, Part 170 is amended as follows:

PART 170—FOOD ADDITIVES

1. The authority citation for 21 CFR Part 170 is revised to read as follows:

Authority: Secs. 201(s), 402, 409, 701(a) (21 U.S.C. 321(s), 342, 348, 371(a)); 21 CFR 5.10.

2. Section 170.3 is amended by revising paragraph (f) to read as follows:

§ 170.3 Definitions.

(f) "Common use in food" means a substantial history of consumption of a substance for food use by a significant number of consumers.

3. Section 170.30 is amended by redesignating paragraph (c) as paragraph (c)(1), by revising the second sentence in paragraph (c)(1), and by adding a new paragraph (c)(2) to read as follows:

§ 170.30 Eligibility for classification as generally recognized as safe (GRAS).

(c)(1) * * * General recognition of safety through experience based on common use in food prior to January 1, 1958, shall be based solely on food use of the substance prior to January 1, 1958, and shall ordinarily be based upon generally available data and information. * * *

(2) A substance used in food prior to January 1, 1958, may be generally recognized as safe through experience based on its common use in food when that use occurred exclusively or primarily outside of the United States if the information about the experience establishes that the use of the substance

is safe within the meaning of the act (see § 170.3(i)). Common use in food prior to January 1, 1958, that occurred outside of the United States shall be documented by published or other information and shall be corroborated by information from a second, independent source that confirms the history and circumstances of use of the substance. The information used to document and to corroborate the history and circumstances of use of the substance must be generally available; that is, it must be widely available in the country in which the history of use has occurred and readily available to interested qualified experts in this country. Persons claiming GRAS status for a substance based on its common use in food outside of the United States should obtain FDA concurrence that the use of the substance is GRAS.

4. Section 170.35 is amended by revising the introductory text of paragraph (c)(1), and by adding a parenthetical phrase at the end of the section to read as follows:

§ 170.35 Affirmation of generally recognized as safe (GRAS) status.

(c)(1) Persons seeking the affirmation of GRAS status of substances as provided in § 170.30(e), except those subject to the NAS/NRC GRAS list survey (36 FR 20546; October 23, 1971), shall submit a petition for GRAS affirmation pursuant to Part 10 of this chapter. Such petition shall contain information to establish that the GRAS criteria as set forth in § 170.30 (b) or (c) have been met, in the following form:

(Collection of information requirements were approved by the Office of Management and Budget (OMB) and assigned OMB control number 0910-0132.)

Dated: May 4, 1988.

John M. Taylor,

Associate Commissioner for Regulatory Affairs.

[FR Doc. 88-10314 Filed 5-9-88; 8:45 am]

BILLING CODE 4160-01-M

DEPARTMENT OF TRANSPORTATION

Coast Guard

33 CFR Part 117

[CGD5-87-063]

Drawbridge Operation Regulations; Pocomoke River, MD

AGENCY: Coast Guard, DOT.

ACTION: Final rule.

SUMMARY: At the requests of the Maryland Department of Transportation and Conrail, the Coast Guard is changing the regulations governing the operation of the Route 675 highway drawbridge across the Pocomoke River, mile 15.8, and adding new regulations for the railroad swing bridge across the Pocomoke River, mile 15.2, at Pocomoke City, Maryland. This action will require five hours advance notice for bridge openings from November 1 to March 31.

EFFECTIVE DATE: These regulations become effective on June 9, 1988.

FOR FURTHER INFORMATION CONTACT: Ann B. Deaton, Bridge Administrator, (804) 398-6222.

SUPPLEMENTARY INFORMATION: On August 18, 1987, the Coast Guard issued a notice of proposed rulemaking concerning this amendment, which was published in the Federal Register on September 4, 1987 (52 FR 34688). Interested persons were given until October 29, 1987, to submit comments on the proposed rule.

The Commander, Fifth Coast Guard District also published the proposal as a Public Notice on November 9, 1987, which gave interested persons until December 18, 1987, to submit comments.

Drafting Information

The drafters of these regulations are Linda L. Gilliam, Project Officer, and CDR Robert J. Reining, Project Attorney.

Discussion of Rule and Comments

The notice of proposed rulemaking would have required vessels to give five hours advance notice for openings between October 1 to March 31 for openings of the railroad swing bridge at mile 15.2 and the Route 675 drawbridge at mile 15.8 across the Pocomoke River. The proposal was intended to eliminate the need to have individuals constantly available to open the draws during a time of year when few vessels transit the river. After the notice of proposed rulemaking was issued, a letter was received from the Mayor of Pocomoke City, dated August 31, 1987. The mayor requested that the proposal be changed to require the Conrail bridge to open on signal from March 1 through October 31. The mayor also stated a general preference for having the bridge open on signal throughout the year.

In addition, in September, 1987, a member of the Fifth Coast Guard District bridge staff was contacted by the DELMARVA Water Transport Committee (DWTC), who stated that they had discussed the issue of manning the bridge during March and October with Conrail. They reported that Conrail did not believe it was feasible to keep

the bridge manned during March, but that Conrail had agreed to leave the railroad bridge manned through the month of October. DWTC, therefore, requested that the proposal be changed to require the bridge to open on signal during a seven month period (April 1 through October 31), rather than the six month period published in the notice of proposed rulemaking (April 1 through September 30).

A member of the Fifth Coast Guard District bridge staff then contacted both Conrail and the Maryland State Highway Administration to obtain their views on the subject. Conrail stated that they had no objections to manning the bridges during the month of October, but they insisted that there was insufficient traffic to keep the bridge manned during the month of March. They requested that they be permitted to only open the draw on five hours notice during March.

On March 18, 1988, the Maryland Department of Transportation stated that they had no objections to the changes proposed by the City for the Route 675 bridge at mile 15.8. No comments were received in response to the notice of proposed rulemaking published in the Federal Register. Only two comments were received as a result of the public notice. One, from a private citizen, favored the proposed regulations. The other, from the U.S. Environmental Protection Agency, Philadelphia, Pennsylvania, stated they had no comments regarding the proposed regulation.

Based on the discussions with Conrail, DWTC, and the Maryland State Highway Administration, we have determined that there is a need to maintain the unrestricted openings of the draws from April 1 through October 31, in order to provide for the reasonable needs of navigation. Therefore, the rule that was originally proposed has been amended to extend the open period from September 30 to October 31.

Good cause exists to issue this final rule without an additional notice of proposed rulemaking, since all the affected interests have been afforded an opportunity to comment on the proposed change and have made their positions known to the Coast Guard. Publication of an additional notice of proposed rulemaking or other public procedures are unnecessary.

Economic Assessment and Certification

These regulations are considered to be non-major under Executive Order 12291 on Federal Regulation and nonsignificant under Department of Transportation regulatory policies and

Recovery would be considered against equity and good conscience.

4. In Part 416, Subpart E, a new § 416.556 is added to read as follows:

§ 416.556 Waiver of adjustment or recovery—countable resources in excess of the limits prescribed in § 416.1205 by \$50 or less.

(a) If any overpayment with respect to an individual (or an individual and his or her spouse if any) is attributable solely to the ownership or possession by the individual (and spouse if any) of countable resources having a value which exceeds the applicable dollar figure specified in § 416.1205 by an amount of \$50.00 or less, including those resources deemed to an individual in accordance with § 416.1202, such individual (and spouse if any) shall be deemed to have been without fault in connection with the overpayment, and waiver of adjustment or recovery will be made, unless the failure to report the value of the excess resources correctly and in a timely manner was willful and knowing.

(b) Failure to report the excess resources correctly and in a timely manner will be considered to be willful and knowing and the individual will be found to be at fault when the evidence clearly shows the individual (and spouse if any) was fully aware of the requirements of the law and of the excess resources and chose to conceal these resources. When an individual incurred a similar overpayment in the past and received an explanation and instructions at the time of the previous overpayment, we will generally find the individual to be at fault. However, in determining whether the individual is at fault, we will consider all aspects of the current and prior overpayment situations, and where we determine the individual is not at fault, we will waive adjustment or recovery of the subsequent overpayment.

[FR Doc. 88-10353 Filed 5-9-88; 8:45 am]

BILLING CODE 4190-11-M

Food and Drug Administration

21 CFR Part 170

[Docket No. 84N-0080]

Eligibility for Classification of Food Substances as Generally Recognized as Safe

AGENCY: Food and Drug Administration.

ACTION: Final rule.

SUMMARY: The Food and Drug Administration (FDA) is amending its regulations to recognize that a substance

that was used in food prior to 1958 can be shown to be generally recognized as safe (GRAS) through experience based on its common use in food outside, as well as in, the United States. This action responds to a court decision that declared invalid an agency regulation that had restricted the experience that could provide the basis for general recognition of safety to experience in the United States. This action also delineates the proof needed to establish that a substance is GRAS on the basis of its foreign use.

DATE: Effective June 9, 1988.

FOR FURTHER INFORMATION CONTACT: Lawrence J. Lin, Center for Food Safety and Applied Nutrition (HFF-334), Food and Drug Administration, 200 C St. SW., Washington, DC 20204, 202-426-8950.

SUPPLEMENTARY INFORMATION:

I. Background

In the Federal Register of July 2, 1985 (50 FR 27294), FDA published a proposal to revise its procedural regulations to establish that a substance in use before 1958 may be eligible for GRAS status based upon its history of use in food outside of the United States. The agency proposed to revise the definitions of "common use in food" in § 170.3(f) (21 CFR 170.3(f)) so that it would no longer stipulate that the history of consumption of the substance must have occurred only in the United States. The agency also proposed to add a new paragraph (c)(2) to § 170.30 that specifies the information that would be required to establish that a substance is GRAS based upon a history of common use in food when that use has occurred outside of the United States. Proposed § 170.30(c)(2) would require verification of the history of common use in food and sufficient information about the use to determine the context in which the use occurred and to establish that use of the substance is safe within the meaning of the Federal Food, Drug, and Cosmetic Act (the Act). The proposed regulation also suggested that persons claiming GRAS status for a substance on the basis of its common use in food outside the United States obtain FDA concurrence that the substance is GRAS.

FDA also proposed to revise the procedures for GRAS affirmation petitions in § 170.35 to make clear that a request for GRAS affirmation may be based upon either scientific procedures or a history of common use in food. The regulation currently stipulates that the petition be based upon scientific procedures.

The proposal responded to a court decision that declared invalid an agency

regulation that had restricted the experience that could provide the basis for a general recognition of safety to experience in the United States.

In § 170.3(f), the agency had defined "common use in food" to mean "a substantial history of consumption of a substance by a significant number of consumers in the United States." On September 15, 1983, however, the United States Court of Appeals for the Ninth Circuit declared § 170.3(f) to be invalid. *Fmali Herb, Inc. v. Heckler*, 715 F.2d 1385 (9th Cir. 1983). The court ruled that by restricting "common use in food" to mean use only in the United States, FDA had imposed a restriction that did not comport with either the literal terms of the act or with the purpose of the common use in food exception, as articulated by the legislators (715 F.2d at 1390).

The agency initially provided 60 days for interested persons to submit written comments on the proposal, but in response to a request from a trade association, the agency extended the period for comment on the proposal for an additional 60 days to November 4, 1985 (50 FR 35571; September 3, 1985).

The agency received 16 comments in response to the proposed regulation. Three comments urged FDA to promulgate the regulation without modification. Two comments noted that the date "January 1, 1985," that appeared in the second column of the Federal Register of July 2, 1985 (50 FR 27297) of the proposed rule should be changed to "January 1, 1958." The agency has already corrected this error in the Federal Register of July 18, 1985 (50 FR 29235). The remaining comments raised the issues that are discussed below.

II. Response to Comments

1. Seven comments objected to the word "solely" in the proposed wording of § 170.3(f), which read: "Common use in food means a substantial history of consumption of a substance solely for food use by a significant number of consumers." These comments stated that the word "solely" in the definition could be interpreted as not allowing GRAS status for food substances that have uses in addition to food uses. One comment stated that FDA is imposing a limitation that is not relevant. The comment stated that the only rationale FDA provided for this limitation is the statement in the preamble that "the information must demonstrate that the substance has in fact been used as a food ingredient and not as a drug, tonic, or folk remedy." Another comment added that in many parts of the world

DEPARTMENT OF HEALTH AND HUMAN SERVICES

FOOD AND DRUG ADMINISTRATION
MEMORANDUM OF TELECONFERENCE

DATE: November 19, 2009

TIME: 1:20 PM

PHONE NUMBER: 541-678-5522

PARTICIPANTS:

FDA

Jeremiah Fasano

HFS-255

External

Robert McQuate

GRAS Associates, LLC

SUBJECT: Status of GRN 295 (*Emblica officinalis* extract)

I contacted Robert McQuate by phone to discuss the status of GRN 295. Dr. McQuate is the agent for Natreon, Inc. (Natreon), the notifier. In a brief phone call on November 16, 2009, we had agreed to discuss FDA's issues with GRN 295 in more detail.

I noted that GRAS notices involving common use in food were very rare in the history of the GRAS program, and that consequently notifiers could not readily look to prior examples of successful GRAS notices of this type. I explained that in our view a GRAS determination based on common use in food needed to observe two "bright lines." The first is that the determination should not rely on any scientific evidence in order to establish its safety. The second is that the evidence of common use should concern food use only. As stated in the Code of Federal Regulations (CFR), "General recognition of safety through experience based on common use in food prior to January 1, 1958, shall be based solely on food use of the substance..." (21 CFR 170.30(c)(1)). I mentioned that two Federal Register (FR) notices issued by FDA in 1986 and 1988 provided useful discussion of the considerations involved in making a GRAS determination based on common use in food prior to 1958, particularly use outside the United States (50 FR 27294 and 53 FR 16544).

Although Natreon's GRAS notice states that its GRAS determination is based on common use in food prior to 1958, the notice included numerous references to medicinal uses and also included supplementary toxicology studies. Thus, the GRAS notice violated both sets of conditions. I explained that our recommendation that Natreon consider

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	(b) (5)								

withdrawing the notice did not mean that we believed it was impossible to make a case for general recognition based on common use in food for this ingredient. Rather, it was our view that this particular version of the notice did not make a narrative argument that was compatible with the constraints of 21 CFR 170.30.

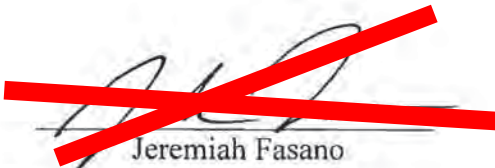
Dr. McQuate explained that both properties of the current notice were based on his concerns about appropriate rigor of the notice. He was concerned that if he omitted discussion of medicinal and therapeutic uses for the plant, we would construe it as an attempt to omit relevant data. He was also concerned that determination of a safe consumption level based on historical extrapolation would be less rigorous than a level based on animal toxicology data.

I explained that in our view discussions of medicinal uses were only relevant to the extent that they raised questions about the safety of an intended food use that might need to be addressed, because history of safe medicinal use does not provide significant insight into safe food use. We appreciated the need to identify the existence of widespread medicinal uses in the service of complete disclosure, but these uses are not pertinent to a food safety assessment and lengthy discussion was not necessary. Documentation of food uses was far more important.

I also noted that we considered that a GRAS determination based on common use had to stand on its own without supporting scientific data, but that it would be acceptable to develop a historical reference exposure based on estimates, extrapolations, and assumptions, as long as the assumptions were plausible and clearly explained, since the notifier would be extrapolating from human population data rather than animal data.

Dr. McQuate expressed his understanding of the points I had raised. I offered to pass on the two FR notices mentioned earlier, which he expressed interest in reading. He stated he would advise his client to withdraw the notification on the understanding that they could resubmit a new notice without prejudice. I suggested that it might be useful for all parties if Dr. McQuate or his client met with FDA prior to resubmission to discuss the exact content of a revised notice, and Dr. McQuate agreed.

We concluded our conversation with the expectation that Dr. McQuate would contact me within a reasonable amount of time with his client's decision.


Jeremiah Fasano

(b) (5)



From: [Fasano, Jeremiah](#)
To: ["mcquate@gras-associates.com"](mailto:mcquate@gras-associates.com);
Subject: Confidential information in GRN 295?
Date: Tuesday, December 08, 2009 11:46:05 AM

Dr. McQuate-

I wanted to confirm my assumption that Natreon does not consider any of the unpublished scientific data in the appendices to GRN 295 confidential. Please let me know if at your convenience if that is not the case.

On a tangential matter, I expect to mail out the official acknowledgement of withdrawal of GRN 295 tomorrow. Please let me know if you do not receive it in a reasonable amount of time.

Regards-

-Jeremiah Fasano

Jeremiah Fasano, Ph.D.
Consumer Safety Officer
Division of Biotechnology and GRAS Notice Review
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
Food and Drug Administration
Phone: 301-436-1173
Fax: 301-436-2964
Email: jeremiah.fasano@fda.hhs.gov

Mailing Address:

HFS-255
5100 Paint Branch Parkway
College Park, MD 20740

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From: [Bob McQuate](#)
To: [Mcmahon, Carrie; Fasano, Jeremiah;](#)
cc: ["Sanni Raju";](#)
Subject: GRN 322 - Cease FDA Review
Date: Wednesday, June 16, 2010 1:45:52 PM
Attachments: [FDA Withdraw Ltr 06 16 10.doc](#)

Dear Dr. McMahon,

Based on your very helpful dialogue of yesterday, I offer the attached letter on behalf of Natreon, Inc. that requests that you cease your evaluation of GRN 322.

Thank you.

Robert S. McQuate, Ph.D.
CEO & Co-Founder
GRAS Associates, LLC
20482 Jacklight Lane
Bend, OR 97702-3074
541-678-5522
mcquate@gras-associates.com
www.gras-associates.com



20482 Jacklight Lane
Bend, OR 97702-3074
541-678-5522
mcquate@gras-associates.com



June 16, 2010

Dr. Jeremiah Fasano
Food and Drug Administration
Center for Food Safety & Applied Nutrition
Office of Food Additive Safety
Division of Biotechnology and GRAS Notice Review (HFS-255)
5100 Paint Branch Parkway
College Park, MD 20740-3835

Re: GRAS Notification 322 --- *Emblica officinalis*

Dear Dr. Fasano:

On behalf of Natreon, Inc., we request that you cease the evaluation of the above-referenced GRAS notice addressing the safety of the aqueous extract of *Emblica officinalis*.

We may elect to resubmit a modified notification at a future date.

Thank you.

Sincerely,

Robert S. McQuate, Ph.D.
CEO & Co-Founder
GRAS Associates, LLC
20482 Jacklight Lane
Bend, OR 97702-3074
541-678-5522
mcquate@gras-associates.com
www.gras-associates.com



April 12, 2010

Robert S. McQuate, Ph.D.
GRAS Associates, LLC
20482 Jacklight Lane
Bend, Oregon
97702-3074

Re: GRAS Notice No. GRN 000322

Dear Dr. McQuate

The Food and Drug Administration (FDA) has received the notice, dated February 1, 2010, that you submitted on behalf of Natreon, Inc. 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 February 5, 2010, filed it on February 18, 2010, and designated it as GRN No. 000322.

The subject of the notice is aqueous extract of *Emblica officinalis*. (*E. officinalis* extract) The notice informs FDA of the view of Natreon, Inc. that *E. officinalis* extract is GRAS, through common use in food, for use as an ingredient in nonalcoholic beverages and processed fruits and fruit juices at levels ranging from 100 to 150 milligrams per serving.

In accordance with proposed 21 CFR 170.36(f), a copy of the information in this notice that conforms to the information described in the proposed GRAS exemption claim (proposed 21 CFR 170.36(c)(1)) is available for public review and copying via the FDA home page at <http://www.fda.gov>. To view or obtain an electronic copy of this information, follow the hyperlinks from the "Food" topic to the "Food Ingredients and Packaging" section to the "Generally Recognized as Safe (GRAS)" page where the GRAS Inventory is listed. If you have any questions about the notice, contact me at jeremiah.fasano@fda.hhs.gov.

Sincerely yours,

Jeremiah Fasano
Division of Biotechnology and
GRAS Notice Review
Center for Food Safety
and Applied Nutrition

DEPARTMENT OF HEALTH AND HUMAN SERVICES

April 12, 2010

Robert S. McQuate, Ph.D.
GRAS Associates, LLC
20482 Jacklight Lane
Bend, Oregon
97702-3074

Re: GRAS Notice No. GRN 000322

Dear Dr. McQuate

The Food and Drug Administration (FDA) has received the notice, dated February 1, 2010, that you submitted on behalf of Natreon, Inc. 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 February 5, 2010, filed it on February 18, 2010, and designated it as GRN No. 000322.

The subject of the notice is aqueous extract of *Emblica officinalis*. (*E. officinalis* extract) The notice informs FDA of the view of Natreon, Inc. that *E. officinalis* extract is GRAS, through common use in food, for use as an ingredient in nonalcoholic beverages and processed fruits and fruit juices at levels ranging from 100 to 150 milligrams per serving.

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Sincerely yours,

Jeremiah Fasano
Division of Biotechnology and
GRAS Notice Review
Center for Food Safety
and Applied Nutrition

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DEPARTMENT OF HEALTH AND HUMAN SERVICES

FOOD AND DRUG ADMINISTRATION
MEMORANDUM OF TELECONFERENCE

DATE: May 27, 2010

TIME: 12 PM

NUMBER: 541-678-5522

PARTICIPANTS:

FDA

Jeremiah Fasano HFS-255

External

Robert McQuate GRAS Associates, LLC

SUBJECT: GRN 322 - Common Use Issues

I called Dr. McQuate at his request to discuss comments I had relayed from the review team on May 26th via email. The review team had concluded that questions remained about the correlation between the identity and use of the subject of GRN 322 and related substances in common use prior to 1958.

Dr. McQuate expressed his disappointment that the changes Natreon had made in response to FDA's comments on an earlier version of their GRAS determination (GRN 295) were not sufficient to answer all our questions. I acknowledged that GRN 322 was a significantly improved submission. I explained the review team's view that the information in the revised notice ultimately did not match the intended substance use provided by Natreon. I noted that the standard for common use in food was challenging in part because many kinds of supporting evidence ordinarily used in a scientific procedures GRAS determination were not available.

Dr. McQuate stated that he would brief Natreon on our discussion. He noted that he was aware of an independent GRAS determination for *Emblica officinalis* aqueous extract at twice the exposure proposed by Natreon. He also noted that he was unsure whether Natreon would choose to re-engage with FDA on the basis of scientific procedures.

Jeremiah Fasano

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Memorandum of Telephone Conversation

Date: June 15, 2010

Between: Carrie McMahon, Ph.D.
and

HFS-255

Robert McQuate, Ph.D.

GRAS Associates, LLC on behalf of
Natreon Inc. (Tel: 541-678-5522)

Subject: aqueous extract of *Emblica officinalis*

Dr. Robert McQuate called to discuss Natreon Inc.'s notice for the use of aqueous extract of *Emblica officinalis*, dated February 1, 2010, and designated GRAS Notice No. GRN 000322 by FDA. Dr. McQuate called in response to an email from Dr. Jeremiah Fasano of FDA, dated May 26, 2010, which provided details of FDA's review of GRN 322.


Dr. McQuate described the bases for the two GRAS notices that he had submitted on behalf of Natreon, Inc. (i.e., GRN 000295 and GRN 000322). He and Dr. McMahon discussed the distinction between the information used to support a GRAS determination based on the common use of a specific "article of commerce" in food pre-1958 in comparison to the information used to support a GRAS determination based on scientific procedures, particularly in relation to ingredients derived from foods with a long history of consumption.

Dr. McMahon reiterated the comments provided by Dr. Fasano, namely, that the history of use prior to 1958 is not robust for the article of commerce (a hot water extract of the fruit *Emblica officinalis*) but that the information provided in the notice about current, long-term uses of the fruit was stronger and could be used to scientifically bridge the chemical identity of the fruit as consumed to the chemical identity of the article of commerce. This bridge could provide the rationale for a GRAS determination based on scientific procedures. For example, once this bridge were established, Dr. McMahon suggested that the EDIs of the safely consumed food could be compared to the EDI for the extract. She also suggested that data and information regarding the toxicity or allergenicity of the constituents of the fruit could be extrapolated to the toxicity/allergenicity assessment of the extract. Finally, Dr. McMahon noted that information, such as scientific studies of the article of commerce or of its constituents, could be included in the narrative as supportive or corroborative.

Dr. McQuate indicated that he understood the comments in Dr. Fasano's email, as discussed by Dr. McMahon. He asked about the best way to proceed (withdrawal and resubmission of a new notice based on scientific procedure or modification of GRN 000322). Dr. McMahon recommended withdrawal as the preferred path forward.


Carrie McMahon, Ph.D.

(b) (5)



From: [Mcmahon, Carrie](#)
To: ["Bob McQuate"; Fasano, Jeremiah;](#)
cc: ["Sanni Raju";](#)
Subject: RE: GRN 322 - Cease FDA Review
Date: Thursday, June 17, 2010 3:25:01 PM

[Dr. McQuate](#) -

I received your email and attachment (both dated June 16, 2010), requesting that we cease review of GRN 322. I have prepared our standard response letter and will put it in the mail shortly. We understand that you may submit a revised notice in the near future and invite you to contact either Dr. Fasano or myself in advance of your submission should you have any questions or wish to discuss your revisions.

Best Regards,

[Carrie](#)

Carrie McMahon, Ph.D.
Consumer Safety Officer
U.S. Food and Drug Administration
Center for Food Safety and Applied Nutrition
Division of Biotechnology and GRAS Notice Review

tel: (301) 436-1202
email: Carrie.McMahon@fda.hhs.gov

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MEMORANDUM OF MEETING

Date: August 26, 2009

Time: 1:00 p.m. – 2:00 p.m.

Location: FDA, Center for Food Safety and Applied Nutrition, Office of Food Additive Safety, 4300 River Road, College Park, MD 20740

Participants:

Visitors:

Sharon Choi, Ph.D.	Cantox Health Sciences International
Melody Harwood, M.Sc.	Cantox Health Sciences International
Kenji Ogimoto	Suika Consulting Office
Shinji Murosaki, Ph.D.	House Wellness Foods Corporation
Yukiko Noguchi	Mitsubishi Corporation

FDA/CFSAN/OFAS/DBGNR:

Vladimir Yurovsky, Ph.D.	HFS-255
Jeanette Glover Glew, M.S.	HFS-255
Edwin Flamm	HFS-255
Susan Carlson, Ph.D.	HFS-255
Ronald Chanderbhan, Ph.D.	HFS-255
Gladys Erives, Ph.D.	HFS-255
Robert L. Martin, Ph.D.	HFS-255
Jannavi Srinivasan, Ph.D.	HFS-255
Shayla West-Barnette, Ph.D.	HFS-255

Subject: Heat-Killed *Lactobacillus plantarum* strain L-137, tradename LP-20

The meeting began with a video overview of House Wellness Foods Corporation (House Wellness). Cantox then discussed the basis for House Wellness' determination that heat-killed *L. plantarum* strain L-137 is GRAS for use in a variety of food and beverages. Cantox stated that House Wellness' GRAS determination is based on information which includes manufacturing, exposure levels, and data and information that support the safety of heat-killed *L. plantarum* strain L-137 (including the safe history of consumption of the bacterial strain).

FDA advised Cantox to discuss the following topics in their submission: 1) whether *L. plantarum* strain L-137 is genetically-engineered; 2) whether *L. plantarum* strain L-137 has been deposited in a culture collection and, if so, provide the deposit number; 3) the purpose of heat-killing the bacterial strain; 4) the procedure used to establish stability

end-points, and 5) whether heat-killed *L. plantarum* strain L-137 is intended for use in infant formulas.


All visiting attendees were given a copy of the attendance sheet prior to their departure.

~~Shayla West-Barnette, Ph.D.~~
Shayla West-Barnette, Ph.D.

ATTACHMENTS

- 1) Request for Pre-Submission Meeting
- 2) Agenda for Pre-Submission Meeting to Discuss the Generally Recognized as Safe (GRAS) Notice for Heat-Killed *Lactobacillus plantarum*
- 3) Information Booklet about House Wellness Foods
- 4) Business Cards for Melody Harwood, Dr. Sharon Choi, Dr. Shinji Murosaki, Yukiko Noguchi, and Kenji Ogimoto

(b) (5)



West-Barnette, Shayla

From: Glew, Jeanette G
Sent: Thursday, July 16, 2009 12:40 PM
To: West-Barnette, Shayla
Subject: FW: Request for Pre-submission Meeting - HK-LP

Attachments: Draft FDA Meeting Agenda HK-LP.doc



Draft FDA Meeting
Agenda HK-LP...

Shayla;

Could you please contact this individual in order to arrange a pre-submission meeting?
Let me know if you have any questions.

Thanks,
JGG

-----Original Message-----

From: Melody Harwood [mailto:mharwood@cantox.com]
Sent: Wednesday, July 15, 2009 5:03 PM
To: Martin, Robert L
Cc: Sharon Choi
Subject: Request for Pre-submission Meeting - HK-LP

Dear Dr. Martin,

I hope this finds you well.

I am contacting you on behalf of our client, House Wellness Foods Corporation (HWFC), who would like to schedule a meeting with representatives of the Administration to go over their self-affirmation of the Generally Recognized As Safe (GRAS) status of their food ingredient, heat-killed *Lactobacillus plantarum* (HK-LP), prior to submitting a GRAS Notification. Below, please find information that might be useful for scheduling the meeting.

- * Name of Company: House Wellness Foods Corporation (Imoji 3-20, Itami, Hyogo, 664-0011, Japan)
- * Anticipated Attendees: Dr. Shinji Murosaki (House Wellness Foods Corporation), Mr. Kenji Ogimoto (SUIKA Consulting), Ms. Yukiko Noguchi (Mitsubishi Corporation), a representative from the Expert Panel (TBD), Dr. Sharon Choi (CANTOX), and myself, Melody Harwood (CANTOX)
- * The product is derived from a non-genetically-modified strain of *Lactobacillus plantarum* (L. plantarum strain L-137).
- * The uses include a variety of traditional food products, including baked goods and baking mixes, beverages and beverage bases, breakfast cereals, dairy product analogs, fats and oils, frozen dairy desserts, grain products and pastas, milk and milk products, plant protein products, processed fruit and fruit juices, processed vegetables and vegetable juices, soft candy, soups and soup mixes, and sugar substitutes at a maximum level of 150 mg per serving.
- * The objectives of the meeting are to provide an overview of the company and the basis for GRAS of HK-LP under the intended conditions of use.
- * Equipment needs: In-focus or other projection machine to connect to a laptop for visual presentation

A draft agenda is attached for your review. Based on the schedules of our client, I would like to propose a meeting on Thursday, 13 August or Friday, 14 August 2009. I do hope that one of these dates will be suitable for yourself and representatives of your department. If not, perhaps another day during the weeks of August 10th or 17th would be possible, so please let me know what alternate dates would be convenient, and I will see if our client can re-arrange their schedule to accommodate.

<<Draft FDA Meeting Agenda HK-LP.doc>>

I would like to thank you in advance for your assistance with this matter. If you require further information, please do not hesitate to contact Dr. Sharon Choi or I by telephone or via email. I look forward to hearing from you at your earliest convenience.

With kindest regards,
Melody

Melody Harwood, M.Sc.
Associate Director, Business Development
Food and Nutrition
Cantox Health Sciences International

2233 Argentia Road, Suite 308
Mississauga, ON, L5N 2X7, CANADA
Tel: 905-542-2900, Extension 302
Fax: 905-542-1011
Mobile: 905-580-6693
mharwood@cantox.com

P Please consider the environment before printing this e-mail.

**PRE-SUBMISSION MEETING TO DISCUSS THE
GENERALLY RECOGNIZED AS SAFE (GRAS) NOTICE FOR
HEAT-KILLED LACTOBACILLUS PLANTARUM**

AGENDA

August 26, 2009
1:00 PM

4300 River Rd.
College Park, MD 20740

Participants: CFSAN Representatives - To be Determined
Dr. Shinji Murosaki, House Wellness Foods Corporation
Mr. Kenji Ogimoto, SUIKA Consulting
Ms. Yukiko Noguchi, Mitsubishi Corporation
Ms. Melody Harwood, Cantox Health Sciences International
Dr. Sharon Choi, Cantox Health Sciences International

- 1. Introductions**
- 2. Overview of House Wellness Foods Corporation**
- 3. Basis for GRAS**
 - (a) Manufacturing, Chemistry, Specifications**
 - (b) Uses and Exposure**
 - (c) Data Supporting GRAS status**
- 4. Next Steps**

House Wellness Foods,
a leading company in Vitamin C drinks,
has produced health-promoting products for half a century.

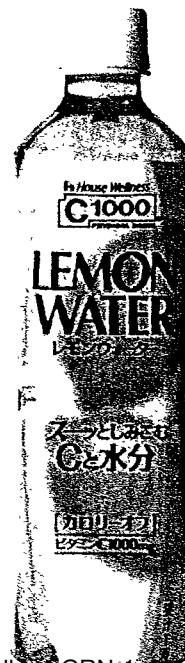
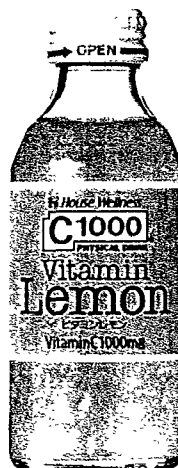
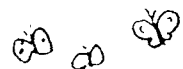
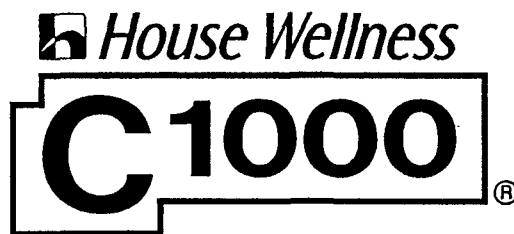
For fifty years since our predecessor, Takeda Food Products Limited, started its production of "PLUSSY" (PLUS"C"), a pioneering Vitamin C beverage product, we have been dedicated to the healthy life of people through the development and production of various functional beverages and health food products.

At the root of our product development lies the concept of "healthier and more delicious".

However healthy the constituents are, if it can't be casually and deliciously taken, it won't last as a health habit. The "C1000" series, our longest-selling product to represent the company since its launch, is indeed the core of our corporate principle.

Our aspiration is making health food products a companion living so close at hand that it's accessible anytime, anywhere, whenever you wish.

In order to materialize that commitment we've held since our foundation, the never-ending challenge of House Wellness Foods Corporation goes on today.




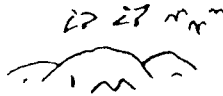



"To serve for a healthier life tomorrow"

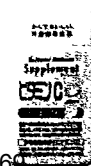
House Wellness Foods was started with the strong aspiration to contribute to the health food product business of House Foods Group, as the "Wellness" in its corporate name embodies. In 2006, House Wellness Foods inherited all the businesses of the former Takeda Food Products Limited, which had supported the food section of Takeda Pharmaceutical Company Limited since 1957. With the foundation of our time-proven knowledge and technology on health and food products, We have delivered health-promoting products for our customers' health, represented by our "C1000 Brand" including "Vitamin Lemon", "Vitamin Water" and "Daily Vitamin", the "Shingen supplement rice" and various other supplements. In product development, with the "devotion to the needs of customers for a healthy dietary life" in mind, we are planning to approach the new needs of "emotional health" involving happiness and liveliness. In addition, we seek to further develop our main line of business, the health food product and beverage business, and expand into the world through our synergy with House Foods Corporation. House Wellness Foods will continue to support your healthy life through providing a better product under our corporate philosophy "To serve for a healthier life tomorrow".

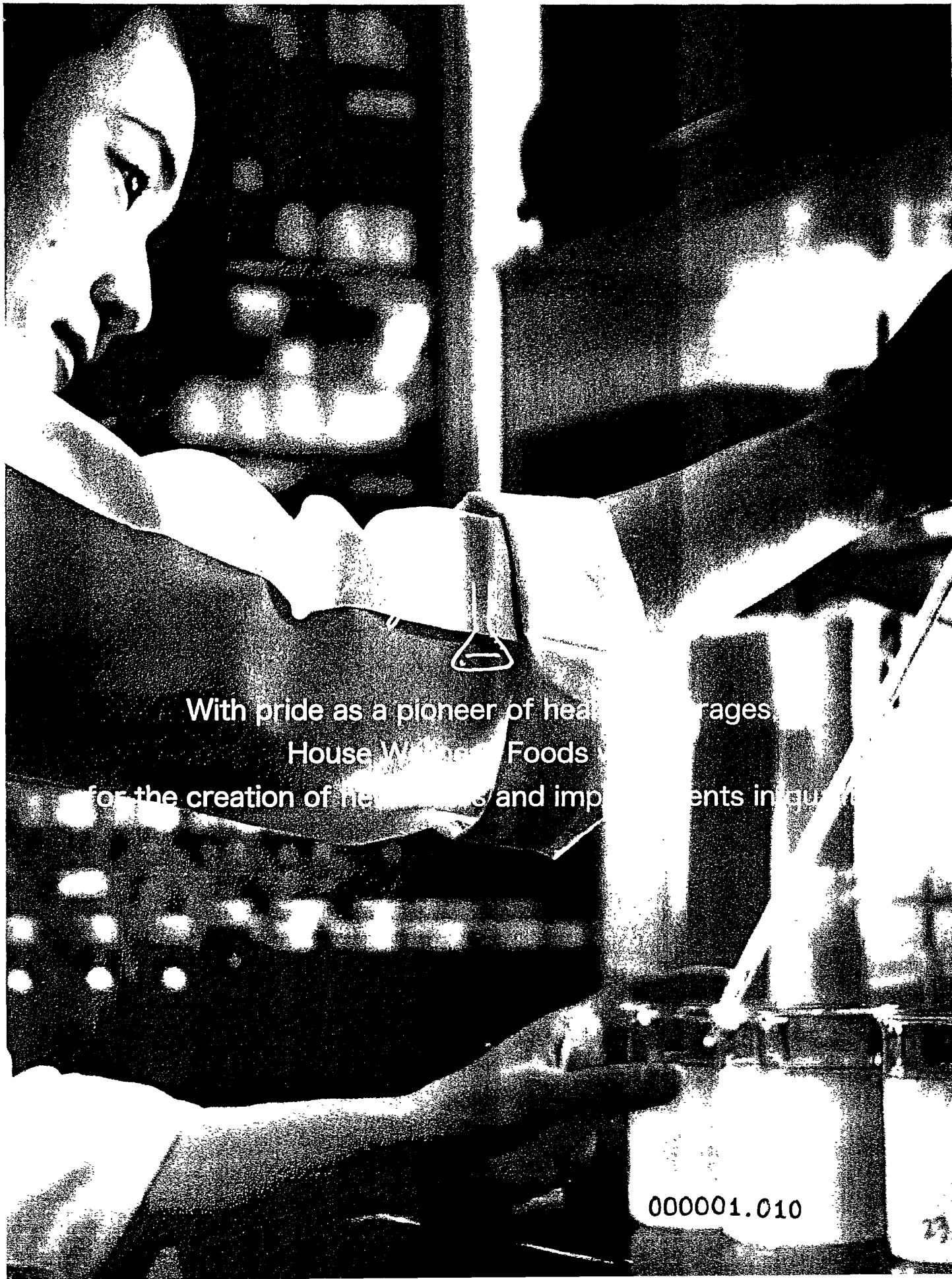
Toshiro Kikuchi, CEO

000001.00

Corporate History

November	1957	Foundation of Takeda Food Products, Ltd.	
April	1958	Production of PLUSSY begins	
December	1960	Production of Ino Ichiban (umami seasoning) begins	
February	1962	Itami Plant completed	
January	1984	Sales & Marketing Division launched ... Production and Sales system established	
February	1990	C1000 Takeda (Fiber & oligo, Fe, Ca, & Mg) goes on the market	
February	1991	C1000 Takeda Vitamin Lemon goes on the market	
September	1994	Better Plus Takeda Vitamin Salad goes on the market	
September	1995	Better Plus Takeda Supplement series goes on the market	
March	1996	Takeda Health Tea goes on the market	
February	1998	PLUSSY Orange goes on the market	
March	1998	C1000 Takeda Vitamin Lemon Jelly goes on the market	
April	1999	Begin stocking and sale of supplemented rice Shingen, Steaming Calcium, and quasi-drug Arinamin series (food products channel) from Takeda Pharmaceutical Company Limited Certified for ISO9001 (Quality Management System) at Production Division for the manufacturing of Ino Ichiban	
September	1999	C1000 Takeda Vitamin Lemon Hot goes on the market	
March	2000	C1000 Takeda Lemon Water 500ml goes on the market	
September	2000	Supplement Takeda Chewable goes on the market	
July	2001	Health supplement Nigero S goes on the market	
September	2001	Takeda Fiber & Peach goes on the market [Ministry of Health, Labor and Welfare certified: Food for specified health use] Supplement Takeda Chewable renewed [Complying with the standards for Food with health claims (Food with nutrient function claims)]	
January	2003	Itami Plant "Bottled beverage production line" redesigned	
June	2003	C1000 Takeda Vitamin Drink Bottle 500ml and C1000 Takeda Lemon Water 900ml go on market	
September	2003	C1000 Takeda Vitamin Lemon Hot PET and Takeda Sarasara Kouka go on the market	
January	2004	Itami Plant "PET bottle beverage production line" completed	
March	2004	C1000 Takeda Vitamin White 500ml goes on the market Takeda Fe Prune 140ml [Complying with the standards for Food with health claims (Food with nutrient function claims)] goes on the market	
April	2004	Certified for ISO14001 (Environmental Management System) at Itami Office	
December	2004	"YOU C1000 Vitamin Lemon" goes on the Indonesian market (Business partnership with P.T. Djojonegoro)	
September	2005	2 products from Takeda Shingen supplement rice (Vitamin+iron added and calcium added) go on the market "General Hygiene Control for Manufacturing Process" for Beverage production line approved and registered	
December	2005	House Foods Corporation and Takeda Pharmaceutical Company Limited announced a business partnership for the beverage and food products business of Takeda Food Products, Ltd.	
March	2006	Certified for ISO9001 (Quality Management System) at Itami Office and entrusted bodies	
April	2006	Foundation of House Wellness Foods Corporation	
June	2006	Fresh Royal Jelly 1000 Drink goes on market	
July	2006	Nigero no Chikara goes on market	
November	2006	C1000 Refresh Lemon & Grapefruit goes on market	
February	2007	C1000 Lemon Water 1L goes on market	
October	2007	Becoming a 100% owned subsidiary of House Foods; Uruoi Shukan 350ml goes on market	
December	2007	Itami Plant "Mini bottled can production line" completed	
March	2008	C1000 Daily Vitamin, C1000 Vitamin Collagen and C1000 Vitamin Collagen Jelly go on the market Business partnership with President Chain Store Corporation (Taiwan) SUPER C Lemon Water 600ml goes on the Taiwanese market	
October	2008	C1000 Concentrate Time goes on the market	
March	2009	C1000 Daily Vitamin Jelly, Asu-e-no Megumi (Yomogi Tea, Soybean Cocoa/FOSHU) goes on the market C1000 Vitamin Lemon Renewal	





With pride as a pioneer of health care, we encourage
Household Foods to
for the creation of new products and improvements in our

000001.010

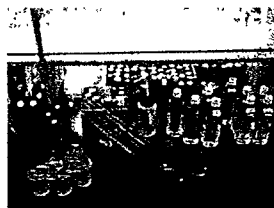
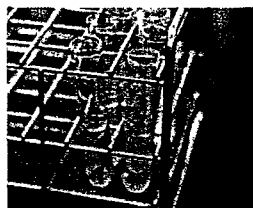
We dedicate ourselves, throughout the production process



from R&D to quality management,
to making a safe and high quality product.



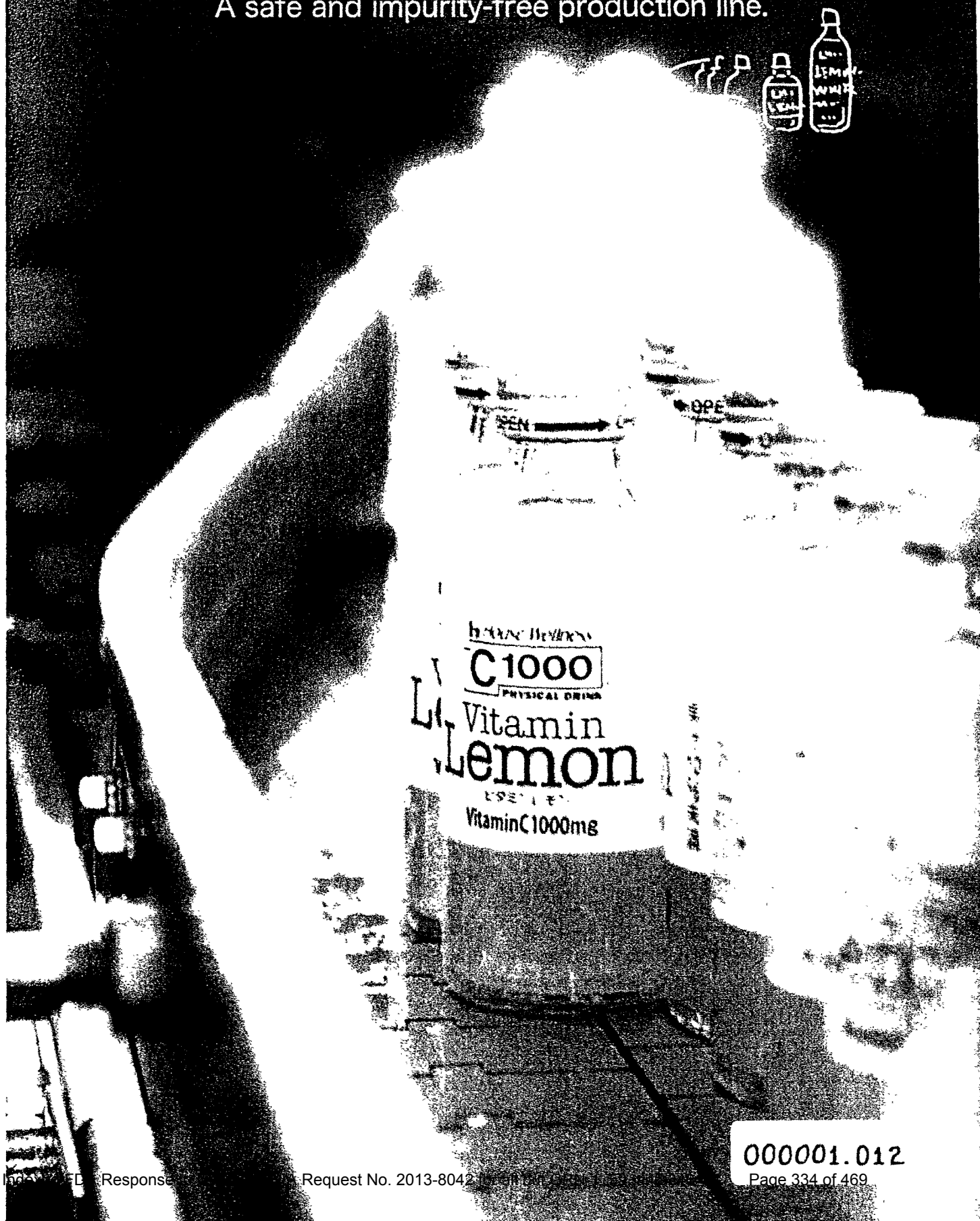
House Wellness Foods is a functional beverage pioneer in Japan. Functional beverages which contain nutrient substances such as vitamins are not uncommon anymore and have become part of our daily lives. We have for over half a century since our production of PLUSSY, a vitamin C-added beverage, and Poly-Rice with added vitamin B₁ as Takeda Food Products, cultivated our vitamin application technology and developed our pharmaceutical knowledge. The R&D team at House Wellness Foods takes part in persistent research with the pride of being a pioneering manufacturer at their



heart. At our R&D Division, research projects that lead to the development of new health-promoting products are underway, utilizing the knowledge and know-how accumulated over the decades. The research findings are also published with relevant academic societies, receiving high acclaim. Meanwhile at the Quality Management Division, we are dedicated to maintaining and improving the quality of our products without overlooking the slightest change, through our advanced examination equipment and the careful eyes of our staff. No minute change should pass here. We understand the responsibility that comes with manufacturing products that are taken into the human body and should complement your health. Today, much public attention is given to the security and safety of food, but our strict basic attitude towards quality control has been consistent and unchanged since our foundation. That is our firm promise to our treasured customers.



From the interchange of human enthusiasm
and leading mechatronics ...
A safe and impurity-free production line.



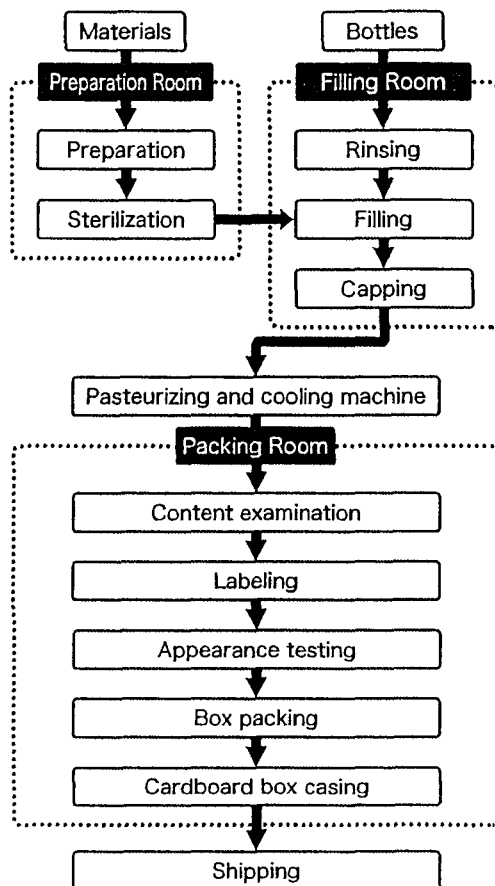
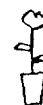
000001.012
Page 334 of 469

From preparation to sterilization, filling and packing ...

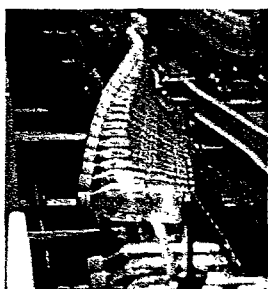
Careful human eyes and our most advanced technology delivers safe,



high-quality products every day.



At our plant located in Itami, Hyogo, near Osaka International Airport, our core products including Vitamin Lemon and Lemon Water are produced 24 hours a day in an impurity-free operation environment. A first-time visitor to our plant may be surprised at how few workers we have in the plant. This is due to our automated production driven by the most advanced mechatronics technology. Most of our manufacturing processes are automated for the

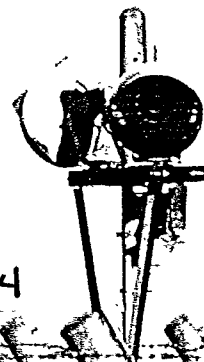


improvement of safety and quality control of the products. Let us introduce the manufacturing processes briefly. Materials carried in are prepared in the Preparation Room and the prepared liquid is sterilized under high temperature here. On the other hand, containers such as glass bottles and PET bottles are brought into the Filling Room in a fully automated process, without coming in contact with humans. Containers are first thoroughly rinsed under high-pressure water by a rinsing device and then filled with the prepared liquid sent from the Preparation Room. Next, it is automatically capped by a capping device. The products will then go through a pasteurization process and sent into the Packing Room. They are then examined inside and out by high-sensitivity sensors and cameras, labeled and packed into cardboard boxes. All these processes are monitored at all times with numerous sensors and cameras. In the case an abnormality is detected, it will be radio communicated to the staff in charge of the production line to be dealt with immediately. What is better for machines to handle is handled by machines and the entire manufacturing processes are monitored by the careful eyes of the staff ... Our production line is firmly supported by the newest technology and the dedication of our staff. Given this, the Itami Plant is certified for ISO9001 and HACCP, international standards for quality and hygiene. Also here at Itami Plant, we recognize the importance of environmental conservation at the regional and global levels, and have acquired the ISO14001 certification by reducing our environmental load, use of energy and resources.



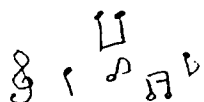


C1000 from Japan to the world ...
The take-off has already begun.



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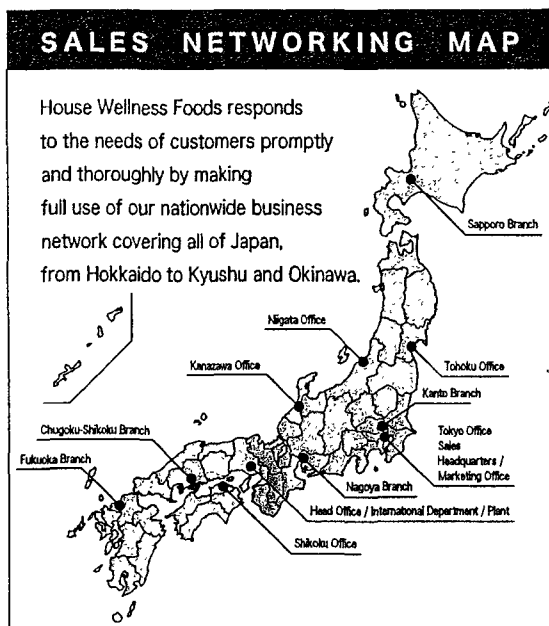
Delivery of "good and healthy products" to other parts of the world ...



International distribution has already taken off,



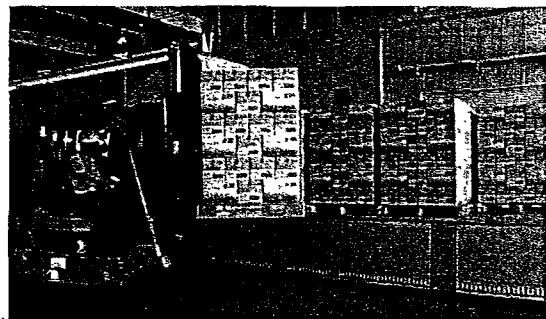
in addition to our business network that covers Japan.



House Wellness Foods has 8 business bases in Japan. Our products are distributed to all parts of Japan and are available at convenience stores, mass merchandise outlets, drug stores, station booths, and vending machines. Our Sales & Marketing staffs catch an array of requests and needs of customers through their contact with customers in these extensive fields. Based on this "live" marketing information, we work on quality

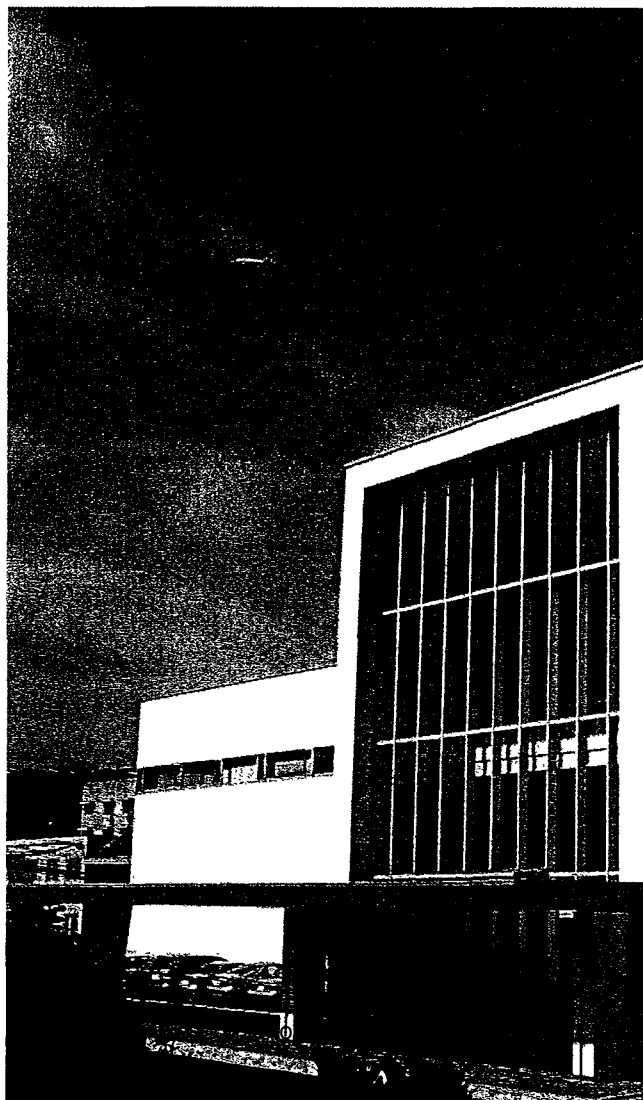


improvements and the development of new products through the collaborative undertakings of the R&D, Quality Management and Production Divisions. In addition, in order to play out our corporate principle to "serve for the healthier life tomorrow" on a bigger stage, we have started our international business expansion. As the first step, House Wellness Foods released supplements in South Korea in 2001. In 2004, the YOU C1000 Vitamin Lemon went on the Indonesian market. In a country where Vitamin C was not regularly taken, the Vitamin Lemon is enjoyed by a wide range of people. In April 2007, the International Department was launched and is operating active business promotions to Indonesia, mainland China, Taiwan, Thailand and the U.S. C1000 from Japan to the world ... Our products are already delivered across the borders to the hands of people who wish for a healthy life.



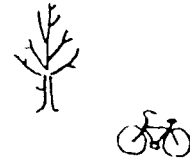
Corporate Profile

Company name	House Wellness Foods Corpora
Head office	3-20 Imoji, Itami, Hyogo
Foundation	April 3, 2006
Capital	100 million yen
Shareholder	House Foods Corporation 100%
Representative	Toshiro Kikuchi, CEO
Number of employees	381 (as of April 1, 2009)
Corporate philosophy	To serve for a healthier life ton
Business lines	Manufacturing and sales of foo feedstuff, feed additives, pet fo
Offices	(Branch) Tohoku, Kanto, Tokyo (Plant) Itami in Hyogo prefectu



■Head Office

3-20 Imoji, Itami, Hyogo 664-0011
TEL.+81-72-778-1121 (pilot number)
FAX.+81-72-772-5155



■International Department, Business Development Division

3-20 Imoji, Itami, Hyogo 664-0011
TEL.+81-72-736-8001 FAX.+81-72-772-5155

■Tokyo Office

Asahi Seimei Sunaga Building 8th Floor,
2-2-6 Bakuro-cho, Nihonbashi, Chuo-ku, Tokyo 103-0002



●Sales Headquarters

●Marketing Office

■Research Institution

●Food Science Research Center

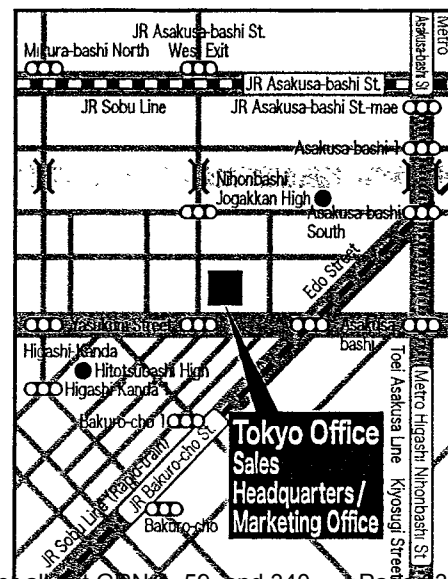
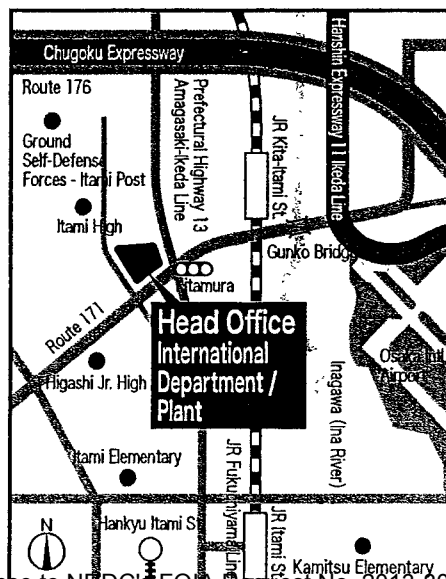
3-20 Imoji, Itami, Hyogo 664-0011
TEL.+81-72-778-1127 FAX.+81-72-778-0892



■Plant

●Itami Plant (Production Division)

3-20 Imoji, Itami, Hyogo 664-0011
TEL.+81-72-778-1123 FAX.+81-72-772-3878





Melody Harwood, M.Sc.
Associate Director
Food & Nutrition Group

2233 Argentia Road, Suite 308
Mississauga, ON, Canada, L5N 2X7
www.cantox.com

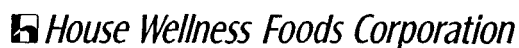
mharwood@cantox.com
Tel: (905) 542-2900
Fax: (905) 542-1011



Sharon Choi, Ph.D.
Scientific and Regulatory Consultant

2233 Argentia Road, Suite 308
Mississauga, ON, Canada, L5N 2X7
www.cantox.com

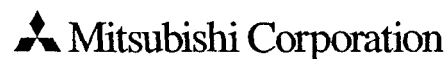
schoi@cantox.com
Tel: (905) 542-2900
Fax: (905) 542-1011



SHINJI MUROSAKI Ph.D.

GENERAL MANAGER
RESEARCH SECTION
FOOD SCIENCE RESEARCH CENTER

IMOJI 3-20, ITAMI, HYOGO, 664-0011, JAPAN
TEL:+81-(0)72-778-1127 FAX:+81-(0)72-778-0892
E-mail: MurosaShinji@house-wf.co.jp



YUKIKO NOGUCHI

LIFE SCIENCE PRODUCTS UNIT
FUNCTIONAL CHEMICALS DIV.

MARUNOUCHI PARK BLDG., 6-1, MARUNOUCHI 2-CHOME,
CHIYODA-KU, TOKYO 100-8086, JAPAN
TEL:81-3-3210-5665 FAX:81-3-3210-5583
E-mail:yukiko.noguchi@mitsubishicorp.com

SUIKA Consulting Office

KENJI OGIMOTO

8-16-19, Sakurayama, Zushi-shi
Kanagawa 249-0005 Japan
TEL:+81-(0)90-3342-1644
FAX:+81-(0)46-873-8773
E-mail:kenji.ogimoto@bloom.ocn.ne.jp



DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
College Park, MD 20740

April 22, 2010

Tetsuya Matsumoto
House Wellness Foods Corporation
International Department
Imoji 3-20, Itami, Hyogo
664-0011
JAPAN

Re: GRAS Notice No. GRN 000324

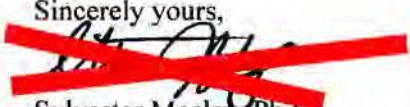
Dear Mr. Matsumoto:

The Food and Drug Administration (FDA) has received the notice, dated January 29, 2010, that you 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)). FDA received this notice on February 5, 2010, filed it on February 18, 2010, and designated it as GRN No. 000324.


The subject of the notice is heat-killed *Lactobacillus plantarum*. The notice informs FDA of the view of House Wellness Foods Corporation that heat-killed *L. plantarum* is GRAS, through scientific procedures, for use as an ingredient in baked goods and baking mixes, beverages and beverage bases, breakfast cereals, dairy product analogs, fats and oils, frozen dairy desserts, grain products and pastas, milk and milk products, plant protein products, processed fruits and fruit juices, processed vegetables and vegetable juices, soft candy, and soups and soup mixes, excluding meat and poultry, at a maximum level of 150 milligram per serving.

In accordance with proposed 21 CFR 170.36(f), a copy of the information in this notice that conforms to the information described in the proposed GRAS exemption claim (proposed 21 CFR 170.36(c)(1)) is available for public review and copying via the FDA home page at <http://www.fda.gov>. To view or obtain an electronic copy of this information, follow the hyperlinks from the "Food" topic to the "Food Ingredients and Packaging" section to the "Generally Recognized as Safe (GRAS)" page where the GRAS Inventory is listed. If you have any questions about the notice, contact me at sylvester.mosley@fda.hhs.gov or 301-436-1333.

Sincerely yours,


Sylvester Mosley, Ph.D.
Division of Biotechnology and
GRAS Notice Review
Center for Food Safety
and Applied Nutrition

(b) (5)



CURRICULUM VITAE OF JOHN BIENENSTOCK, CM, MD (Hon), FRCP, FRCP(C), FRS(C)

CURRENT POSITION: Distinguished University Professor
Director McMaster Brain-Body Institute
St. Joseph's HealthCare Hamilton
Departments of Medicine and
Pathology & Molecular Medicine

DATE AND PLACE OF BIRTH:

(b) (6)

PRESENT ADDRESS:

The McMaster Brain-Body Institute
St. Joseph's Healthcare Hamilton
Juravinski Tower, Room T3304
50 Charlton Avenue East
Hamilton, ON L8N 4A6
Telephone: 905-522-1155 Ext 35203
Fax: 905-540-6593
E-mail: <bienens@mcmaster.ca>

HOME ADDRESS:

(b) (6)

EDUCATION:

1980	F.R.C.P.
1976	F.R.C.P.(C)
1964	M.R.C.P. Royal College of Physicians, London, U.K.
1960	M.B.B.S. King's College and Westminster Hospital Medical School, London, U.K.
	MRCS, LRCP

CLINICAL TRAINING:

1964	Royal Masonic Hospital, London Registrar
1963-64	Royal Free Hospital (Renal Unit), London Senior House Officer to Professor Sherlock
1961-63	St. James Hospital, Balham, London House Physician
1961	St. Mary Abbot's Hospital, London House Physician
1960	Westminster Hospital, London, House Surgeon

POSITIONS HELD:

1964-66	Massachusetts General Hospital, Harvard Medical School, U.S.A. Clinical and Research Fellow
1966	Buffalo General Hospital, State University of New York at Buffalo, U.S.A. Instructor in Medicine
1966-67	Buswell Fellow in Medicine
1967-68	Assistant Research Professor of Medicine
1968-70	McMaster University, Hamilton, Ontario, Canada Assistant Professor in Medicine
1969-74	Canadian Medical Research Council Scholar
1970-74	McMaster University, Hamilton, Ontario, Canada Associate Professor of Medicine
1970-74	Associate Professor of Pathology
1971-78	Director, Host Resistance Programme
1974	Professor of Medicine
1974	Professor of Pathology
1968-86	Co-ordinator, Immunology Programme
1978-89	Chairman, Department of Pathology
1989-97	Vice-President, Faculty of Health Sciences
1991-97	Dean, Faculty of Health Sciences
2001-	Director, Brain-Body Institute

OTHER:

1968-69	St. Joseph's Hospital, Hamilton, Ontario, Canada Associate staff
1971	McMaster University Medical Centre and Chedoke Hospital, Hamilton Active Staff
1974-77	Ward 4C and Outpatients 2F SAS Area (combined Rheumatology/Orthopedics, Immunology) Unit Director
1979-80	Chester Beatty Research Institute, Fulham Road, London, U.K. Haddow Fellow with Professor A.J.S. Davies (Sabbatical Year)
1987	Departments of Pathology and Microbiology, University of Western Australia Rayne Visiting Professor (one month)

COMMITTEES:

McMaster University

University Senate (1971-75)
President's Budgetary Advisory (1973-75)
President's Executive (1989-96)
Board of Governors (1989-96)

Faculty Committees:

Graduate Curriculum and Policy (1970-73, 89)

Updated May 26, 2010

Graduate Studies and Admission (1970-73)

Faculty Executive, Chair (1972-96)

Faculty Council (1972-96)

Appointments (1973-79)

Promotions and Tenure (1973-79)

Chair (1993)

Committee for Scientific Development

(Associate Dean for Research)

Member (1970-72)

Chair (1972-78)

Finance Committee

Capital Equipment Review (Hospital equipment budget)

Chairman (1978-80)

District Committee for Laboratory Medicine (1973-89)

Chairman, Advisory Committee (1978-89)

Chedoke-McMaster Hospitals

Medical Advisory Committee (1978-89)

Board of Trustees (1989-96)

Foundation (1989-96)

Hospital Executive (1990-96)

St. Joseph's Hospital Board (1993-96)

Provincial (Ontario)

Health Research and Development Committee (1974-79)

Council of Ontario Faculties of Medicine (Deans), Chair (1995-96)

John Charles Polanyi Prizes Selection Committee (2001-04)

Federal

Canadian Medical Research Council Committee of the uses of antilymphocyte serum other than for transplantation

Canadian Medical Research Council (1970)

Grants Committee for Immunology and Transplantation

Member (1969-75)

Chairman (1973-75, 1977-78)

National

Canadian Society of Immunology

Councillor (1971-73)

Secretary (1973-75)

President (1985-87)

Royal College of Physicians and Surgeons of Canada

Committee on Clinical Immunology

Corresponding Member (1976-80)

Canadian Red Cross Society

National Blood Transfusion Service

Advisory Committee Member

Chairman (1985-88)

Updated May 26, 2010

Research and Development Sub-Committee

Chairman (1982-85)

Blood Services Committee

Chairman (1988-90)

Board of Governors

Member (1985-90)

Canadian Arthritis and Rheumatism Society

Research Projects Panel

Chairman (1988-90)

Medical Planning Committee

Member

Royal Society of Canada

Membership Nomination Committee (Life Sciences) (1993-95)

Scientific Advisory Board, Beacon Diagnostics Inc. (U.S.A.) (1996)

International

Collegium Internationale Allergologicum

Executive Secretary/Treasurer (1987-94)

President Elect (1994-98)

President (1999-2003)

World Health Organization

Steering Committee: Encapsulated Bacteria

Consultant (1985-90)

International Union of Immunological Societies (1989-92)

Councillor Committee on Standardization

Member (1971-72)

International Review Panel of the Western Australian Research Institute for Child Health

Member (1989-)

International Society for Neuroimmunomodulation

Advisory Board Member (1990-)

Society for Mucosal Immunology

President (1990-92)

Other

Dundas Valley School of Art

Board of Governors (1978-)

Chairman of the Board (1984-86)

Art Gallery of Hamilton

Board Member (1990-93)

American Biographical Institute, Inc.

Research Board of Advisors (1999-)

HONOURS

Canadian MRC Scholarship (1969-74)

Canadian Association of Gastroenterology (1983)

Research Lecturer

Faculty of Medicine, State University of New York, Buffalo

D.W. Harrington Lectureship (1986)

Updated May 26, 2010

Swiss Society of Allergology and Immunology (1987)

Honorary Member

American Association for the Advancement of Science (1987)

Fellow

Association of American Physicians

Member (1987)

Ottawa Civic Hospital, Loeb Institute

Scientific Advisory Committee (1987-90)

Pharmacia Allergy Research Foundation (1987-89)

Scientific Board

Honorable Membership, Pharmacia Allergy Research Foundation (2000)

Faculty of Medicine, University of Nebraska, Omaha, Nebraska

Ross A. McIntyre Award (1989)

Association of Czechoslovak Societies of Medicine, Purkynje Medal, Prague, Czechoslovakia (1989)

Member, Order of the Red Cross (1990)

Fellow, The Royal Society of Canada (1992)

Fellow, King's Fund International (1995)

Volunteer Service Award, Ontario (1992)

Ministry of Citizenship and Culture and Communication

The Commemorative Medal for the 125th Anniversary of the Confederation of Canada (1992)

The University of Texas Medical Branch, Galveston, Texas

James W. McLaughlin Fellowship Visiting Professor

Finkelstein Prize, Crohn's and Colitis Foundation of Canada (1996)

Distinguished University Professor, McMaster University (1997)

Doctor Honoris Causa in Medicine, Göteborg, Sweden (1998)

Scientific Achievement Award, International Association of Allergy and Clinical Immunology (2000)

Member, Order of Canada (2002)

Governor General of Canada Golden Jubilee Medal (2002)

Honorary Member, German Society for Allergy & Clinical Immunology (2004)

Who's Who in America, Canadian Who's Who, Who's Who in Ontario, International Who's Who, Who's Who in Science and Engineering, American Men and Women of Science, International Directory of Distinguished Leadership, International Who's Who of Contemporary Achievement, Who's Who in Medicine and Health Care, American Biographical Institute's Five Thousand Personalities of the World, Who's Who's 2000 Outstanding Scientists of the 20th Century, Five Thousand Personalities of the World, Millennium Hall of Fame

CONSULTANCIES

Roussel UCLAF, Paris, France

Nippon Zoki, Osaka, Japan

MDS, Toronto, Canada

Connaught, Labs, Toronto, Canada

CIBA-GEIGY, Basel, Switzerland

Upjohn, Kalamazoo, U.S.A.

ICI, Unilever, U.K.

Report of an External Review of the National Health and Medical Research Council (NHMRC) – Australia (1993)

The University of British Columbia, Canada – Review of the Faculty of Medicine (1995)

The University of Sydney, Australia – Research Review of the Faculty of Medicine (1995)

Göteborg University, Sweden – Review on the Research of the Faculty of Medicine (1996)

Chief Scientific Officer, OraTol, U.K. (1998-1999)

Updated May 26, 2010

Chair, Scientific Advisory Board, IMI (International Medical Innovations), Canada (1998-2008)

Director, ProMetic Life Sciences, Inc., Canada (1999-)

Karolinska Institute, Sweden— Chair, Review of Public Health Programs (2000)

Chair, Scientific Advisory Board, VRI BioMedical, Australia (2001-2004)

Lallemand Institut Rosell, Science Advisory Board, 2009-

FOUNDING MEMBER

AB Biological Supplies, Inc. (1978)

Agritech, Inc. (1980)

ASSOCIATIONS AND SOCIETIES

Memberships

American Academy of Allergy and Immunology (Fellow)

American Association for the Advancement of Science (Fellow)

American Association of Immunologists

American Association of Pathologists

American Federation for Clinical Research

American Rheumatism Association

American Society for Clinical Investigation (1975)

American Thoracic Society

Association of American Physicians (1987)

British Society of Immunology

Canadian Arthritis and Rheumatism Society

Canadian Federation of Biological Societies

Canadian Institute of Academic Medicine

Canadian Society for Clinical Investigation

Canadian Thoracic Society

Clinical Immunology Society

Collegium Internationale Allergologicum

Hamilton Academy of Medicine

Hamilton Medical-Legal Society

New York Academy of Sciences

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DR. JOHN BIENENSTOCK
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172. Bienenstock, J (2002): Stress and Asthma: The Plot Thickens (Editorial) *Am J Respir Crit Care* 165:1034-5
173. Van der Kleij H, Blennerhassett MG, Bienenstock J. (2003): Mast cells and nerve interaction. In: *Autonomic Immunology*. Ed. by Blennerhassett MG, Goetzl E, Bienenstock J. Taylor and Francis, London, pp.139-170.
174. Ma D, Bienenstock J. (2003): Role of epithelium-environment interactions in down-regulation of mucosal inflammation. In: *Gastrointestinal Inflammation and Disturbed Gut Function*. Falk Symposium 130. Ed. by Holtmann G and Talley NJ, Kluwer Academic Publishers, Germany.
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176. van der Kleij HPM, Blennerhassett MG, Bienenstock J. (2004): Two-way communication between mast cells and the nervous system. Chapter 18, 357-380, in *Neuro-immunoendocrinology* (Eds. V. Geenen & R. Johnson) Marcel Dekker, New York
177. van der Kleij H, Bienenstock J. (2006): Significance of sensory neuropeptides and the immune response. 4th Edition, Volume 1, Chapter 4, 97-129, in *Psychoneuroimmunology* (Eds. R. Ader), Elsevier Academic Press, London.

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179. Forsythe P, Bienenstock J (2009) Neural regulation of the immune response. In: Allergy Frontiers: Epigenetics to Future Perspectives: Volume 2: (S. Holgate, R. Pawankar and L. Rosenwasser, eds) 493:514

Books Edited:

1. Immunology of the Lung and Upper Respiratory Tract, (ed. Bienenstock J) McGraw Hill, New York, 1984.
2. Mast Cell Differentiation and Heterogeneity, (eds. Befus AD, Bienenstock J and Denburg JA) Raven Press, New York, 1986.
3. Recent Advances in Mucosal Immunology - Part A and B, (eds. Mestecky J, McGhee JR, Bienenstock J and Ogra PL) Plenum Press, New York, 1987.
4. Handbook of Mucosal Immunology, (eds. Ogra PL, Lamm ME, McGhee JR, Mestecky J, Strober W, and Bienenstock J) Academic Press, New York, 1993.
5. Handbook of Mucosal Immunology, (eds. Ogra PL, Lamm ME, McGhee JR, Mestecky J, Strober W, and Bienenstock J) Academic Press, New York, 1998.
6. Autonomic Neuroimmunology. (eds. Blennerhassett MG, Goetzel E, Bienenstock J) Taylor and Francis, London, 2003.
7. Allergy Frontiers and Futures, (eds. Bienenstock J, Ring J, and Togias, A.G.) Hogrefe & Huber, Gottingen, Germany, 2004
8. Mucosal Immunology (3rd edition, Volumes I and II). (eds. Mestecky J, Lamm ME, Strober W, Bienenstock J, Mayer L, McGhee JR. Elsevier, UK 2005.
9. From Genes to Phenotypes: The basis of Future Allergy Management, (eds. Lowenstein H, Bienenstock J, and Ring J) Hogrefe & Huber, Gottingen, Germany, 2005

Dr. John Bienenstock: Biosketch –May 2010

Dr. John Bienenstock is internationally known as a physician and mucosal immunologist. He trained at King's College, London and Westminster Hospital, London, U.K. He holds the title of Distinguished University Professor at McMaster University, an Honorary MD (Goteborg, Sweden), is a Fellow of the Royal Society of Canada and a Member of the Order of Canada. He is the Founding Director of the McMaster Brain-Body Institute at St. Joseph's Healthcare Hamilton, a former Chair of Pathology and subsequently Dean and Vice-President of the Faculty of Health Sciences, McMaster University.

Dr. Bienenstock is a frequent speaker at symposia and plenary sessions and has organized a number of international conferences. He has served as the President of the Canadian Society of Immunology, the Society of Mucosal Immunology and the Collegium Internationale Allergologicum. He was invited by the Government of Australia to review the operation of the National Health and Medical Research Council. This resulted in the overhaul and restructuring of the Council's organization and operations. He similarly reviewed the research operations of the Universities of Sydney, Australia, and Goteborg, Sweden and in 2000 completed a review of the Faculty of Public Health for the Karolinska Institute, Stockholm, Sweden. He acts as a consultant to venture capital groups, foundations and the pharmaceutical industry.

He has held a continuous operating research grant from the Medical Research Council of Canada for more than 20 years. He has published more than 500 peer reviewed articles and other publications. He has authored, edited and co-edited 8 books including the standard text on mucosal immunology. He has supervised some 54 post doctoral fellows and 10 doctoral students.

His areas of interest are: immunophysiology; mucosal immunology and its alteration in a variety of disease models; mast cell biology; the role of neuroimmune interactions in allergy and inflammation; the reciprocal communication between the nervous system and immune systems; mechanisms of action of commensal bacteria on the nervous system and behaviour and in various models of inflammation.

CURRICULUM VITAE OF JOHN BIENENSTOCK, CM, MD (Hon), FRCP, FRCP(C), FRS(C)

CURRENT POSITION: Distinguished University Professor
Director McMaster Brain-Body Institute
St. Joseph's HealthCare Hamilton
Departments of Medicine and
Pathology & Molecular Medicine

DATE AND PLACE OF BIRTH:

(b) (6)

PRESENT ADDRESS:

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St. Joseph's Healthcare Hamilton
Juravinski Tower, Room T3304
50 Charlton Avenue East
Hamilton, ON L8N 4A6
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HOME ADDRESS:

(b) (6)

EDUCATION:

1980	F.R.C.P.
1976	F.R.C.P.(C)
1964	M.R.C.P. Royal College of Physicians, London, U.K.
1960	M.B.B.S. King's College and Westminster Hospital Medical School, London, U.K. MRCS, LRCP

CLINICAL TRAINING:

1964	Royal Masonic Hospital, London Registrar
1963-64	Royal Free Hospital (Renal Unit), London Senior House Officer to Professor Sherlock
1961-63	St. James Hospital, Balham, London House Physician
1961	St. Mary Abbot's Hospital, London House Physician
1960	Westminster Hospital, London, House Surgeon

POSITIONS HELD:

1964-66	Massachusetts General Hospital, Harvard Medical School, U.S.A. Clinical and Research Fellow
1966	Buffalo General Hospital, State University of New York at Buffalo, U.S.A. Instructor in Medicine
1966-67	Buswell Fellow in Medicine
1967-68	Assistant Research Professor of Medicine
1968-70	McMaster University, Hamilton, Ontario, Canada Assistant Professor in Medicine
1969-74	Canadian Medical Research Council Scholar
1970-74	McMaster University, Hamilton, Ontario, Canada Associate Professor of Medicine
1970-74	Associate Professor of Pathology
1971-78	Director, Host Resistance Programme
1974	Professor of Medicine
1974	Professor of Pathology
1968-86	Co-ordinator, Immunology Programme
1978-89	Chairman, Department of Pathology
1989-97	Vice-President, Faculty of Health Sciences
1991-97	Dean, Faculty of Health Sciences
2001-	Director, Brain-Body Institute

OTHER:

1968-69	St. Joseph's Hospital, Hamilton, Ontario, Canada Associate staff
1971	McMaster University Medical Centre and Chedoke Hospital, Hamilton Active Staff
1974-77	Ward 4C and Outpatients 2F SAS Area (combined Rheumatology/Orthopedics, Immunology) Unit Director
1979-80	Chester Beatty Research Institute, Fulham Road, London, U.K. Haddow Fellow with Professor A.J.S. Davies (Sabbatical Year)
1987	Departments of Pathology and Microbiology, University of Western Australia Rayne Visiting Professor (one month)

COMMITTEES:

McMaster University

- University Senate (1971-75)
- President's Budgetary Advisory (1973-75)
- President's Executive (1989-96)
- Board of Governors (1989-96)

Faculty Committees:

- Graduate Curriculum and Policy (1970-73, 89)

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Graduate Studies and Admission (1970-73)

Faculty Executive, Chair (1972-96)

Faculty Council (1972-96)

Appointments (1973-79)

Promotions and Tenure (1973-79)

Chair (1993)

Committee for Scientific Development

(Associate Dean for Research)

Member (1970-72)

Chair (1972-78)

Finance Committee

Capital Equipment Review (Hospital equipment budget)

Chairman (1978-80)

District Committee for Laboratory Medicine (1973-89)

Chairman, Advisory Committee (1978-89)

Chedoke-McMaster Hospitals

Medical Advisory Committee (1978-89)

Board of Trustees (1989-96)

Foundation (1989-96)

Hospital Executive (1990-96)

St. Joseph's Hospital Board (1993-96)

Provincial (Ontario)

Health Research and Development Committee (1974-79)

Council of Ontario Faculties of Medicine (Deans), Chair (1995-96)

John Charles Polanyi Prizes Selection Committee (2001-04)

Federal

Canadian Medical Research Council Committee of the uses of antilymphocyte serum other than for transplantation

Canadian Medical Research Council (1970)

Grants Committee for Immunology and Transplantation

Member (1969-75)

Chairman (1973-75, 1977-78)

National

Canadian Society of Immunology

Councillor (1971-73)

Secretary (1973-75)

President (1985-87)

Royal College of Physicians and Surgeons of Canada

Committee on Clinical Immunology

Corresponding Member (1976-80)

Canadian Red Cross Society

National Blood Transfusion Service

Advisory Committee Member

Chairman (1985-88)

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Research and Development Sub-Committee

Chairman (1982-85)

Blood Services Committee

Chairman (1988-90)

Board of Governors

Member (1985-90)

Canadian Arthritis and Rheumatism Society

Research Projects Panel

Chairman (1988-90)

Medical Planning Committee

Member

Royal Society of Canada

Membership Nomination Committee (Life Sciences) (1993-95)

Scientific Advisory Board, Beacon Diagnostics Inc. (U.S.A.) (1996)

International

Collegium Internationale Allergologicum

Executive Secretary/Treasurer (1987-94)

President Elect (1994-98)

President (1999-2003)

World Health Organization

Steering Committee: Encapsulated Bacteria

Consultant (1985-90)

International Union of Immunological Societies (1989-92)

Councillor Committee on Standardization

Member (1971-72)

International Review Panel of the Western Australian Research Institute for Child Health

Member (1989-)

International Society for Neuroimmunomodulation

Advisory Board Member (1990-)

Society for Mucosal Immunology

President (1990-92)

Other

Dundas Valley School of Art

Board of Governors (1978-)

Chairman of the Board (1984-86)

Art Gallery of Hamilton

Board Member (1990-93)

American Biographical Institute, Inc.

Research Board of Advisors (1999-)

HONOURS

Canadian MRC Scholarship (1969-74)

Canadian Association of Gastroenterology (1983)

Research Lecturer

Faculty of Medicine, State University of New York, Buffalo

D.W. Harrington Lectureship (1986)

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Swiss Society of Allergology and Immunology (1987)

Honorary Member

American Association for the Advancement of Science (1987)

Fellow

Association of American Physicians

Member (1987)

Ottawa Civic Hospital, Loeb Institute

Scientific Advisory Committee (1987-90)

Pharmacia Allergy Research Foundation (1987-89)

Scientific Board

Honorable Membership, Pharmacia Allergy Research Foundation (2000)

Faculty of Medicine, University of Nebraska, Omaha, Nebraska

Ross A. McIntyre Award (1989)

Association of Czechoslovak Societies of Medicine, Purkynje Medal, Prague, Czechoslovakia (1989)

Member, Order of the Red Cross (1990)

Fellow, The Royal Society of Canada (1992)

Fellow, King's Fund International (1995)

Volunteer Service Award, Ontario (1992)

Ministry of Citizenship and Culture and Communication

The Commemorative Medal for the 125th Anniversary of the Confederation of Canada (1992)

The University of Texas Medical Branch, Galveston, Texas

James W. McLaughlin Fellowship Visiting Professor

Finkelstein Prize, Crohn's and Colitis Foundation of Canada (1996)

Distinguished University Professor, McMaster University (1997)

Doctor Honoris Causa in Medicine, Göteborg, Sweden (1998)

Scientific Achievement Award, International Association of Allergy and Clinical Immunology (2000)

Member, Order of Canada (2002)

Governor General of Canada Golden Jubilee Medal (2002)

Honourary Member, German Society for Allergy & Clinical Immunology (2004)

Who's Who in America, Canadian Who's Who, Who's Who in Ontario, International Who's Who, Who's Who in Science and Engineering, American Men and Women of Science, International Directory of Distinguished Leadership, International Who's Who of Contemporary Achievement, Who's Who in Medicine and Health Care, American Biographical Institute's Five Thousand Personalities of the World, Who's Who's 2000 Outstanding Scientists of the 20th Century, Five Thousand Personalities of the World, Millennium Hall of Fame

CONSULTANCIES

Roussel UCLAF, Paris, France

Nippon Zoki, Osaka, Japan

MDS, Toronto, Canada

Connaught, Labs, Toronto, Canada

CIBA-GEIGY, Basel, Switzerland

Upjohn, Kalamazoo, U.S.A.

ICI, Unilever, U.K.

Report of an External Review of the National Health and Medical Research Council (NHMRC) – Australia (1993)

The University of British Columbia, Canada – Review of the Faculty of Medicine (1995)

The University of Sydney, Australia – Research Review of the Faculty of Medicine (1995)

Göteborg University, Sweden – Review on the Research of the Faculty of Medicine (1996)

Chief Scientific Officer, OraTol, U.K. (1998-1999)

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Chair, Scientific Advisory Board, IMI (International Medical Innovations), Canada (1998-2008)

Director, ProMetic Life Sciences, Inc., Canada (1999-)

Karolinska Institute, Sweden— Chair, Review of Public Health Programs (2000)

Chair, Scientific Advisory Board, VRI BioMedical, Australia (2001-2004)

Lallemand Institut Rosell, Science Advisory Board, 2009-

FOUNDING MEMBER

AB Biological Supplies, Inc. (1978)

Agritech, Inc. (1980)

ASSOCIATIONS AND SOCIETIES

Memberships

American Academy of Allergy and Immunology (Fellow)

American Association for the Advancement of Science (Fellow)

American Association of Immunologists

American Association of Pathologists

American Federation for Clinical Research

American Rheumatism Association

American Society for Clinical Investigation (1975)

American Thoracic Society

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DR. JOHN BIENENSTOCK
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Memorandum of Telephone Conversations

Date: April 28 and 29, 2010

Between:

Ashley Roberts, Ph.D.	Cantox Health Sciences International (905) 542-2900
Sharon Choi, Ph.D.	Cantox Health Sciences International

And

Edwin Flamm	HFS-255
Jannavi Srinivasan, Ph.D.	HFS-255
Jeremiah Fasano, Ph.D.	HFS-255
Michael DiNovi, Ph.D.	HFS-255
Paulette Gaynor, Ph.D.	HFS-255
Robert Merker, Ph.D.	HFS-265
Ronald Chanderbhan, Ph.D.	HFS-255
Sylvester Mosley, Ph.D.	HFS-255
Vladimir Yurovsky, Ph.D.	HFS-255

Subject: Deficiencies for GRN000324 (Heat-killed *Lactobacillus plantarum*)

At a pre-arranged time on April 28, 2010, Cantox conducted the telephone call on behalf of the notifier (House Wellness Foods Corporation). Following introductions, FDA personnel opened by stating that as a result of our review, several questions or deficiencies were uncovered. These questions or deficiencies were in three overarching areas (microbiology, exposure, and immunology) and included points for clarification.

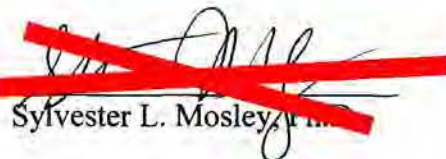
FDA reviewers then discussed the technical questions or deficiencies noted during their reviews. Subsequent to the discussion, FDA sent a list of issues by electronic mail (see attachment).

FDA personnel then discussed administrative details, noting that as of today, April 28, 2010, the review process of GRN000324 is on day 70 and that as a program goal, the review process of most Generally Recognized as Safe (GRAS) notices should be completed within 180 days. Furthermore, because of the three overarching areas as well as the other points for clarification that had been raised, it is highly unlikely that comprehensive edits can be made within an appropriate time-frame. FDA personnel then stated that the notice in its current state, including the amendments, would not get to a "no questions" response from FDA. FDA personnel discussed the following two options with Cantox:

1. Withdraw the notice without prejudice. This will allow the notifier time to address the team's questions and then come back to us when the deficiencies have been addressed. FDA personnel are more than willing to review this information prior to the notifier resubmitting the notice.
2. If the notifier feels the data is readily available and will sufficiently address the deficiencies discussed and the notifier can get this information to us within a two week time period, then FDA could continue the review of this notice when this information is received.

After hearing these options, Cantox stated they will take a look at the questions when they receive them and let FDA know of the notifier's decision.


On April 29, 2010, Drs. Chanderbhan and DiNovi called Dr. Roberts of Cantox to discuss the immunology issues associated with their client's GRAS notice. Drs. Chanderbhan and DiNovi stressed to Cantox the importance that the notifier have a scientist qualified to speak to the immunological issues that were raised.


Sylvester L. Mosley

Attachment:

1. Electronic mail message dated May 4, 2010, from Sylvester Mosley of FDA to Ashley Robert and Sharon Choi of Cantox Health Sciences International

(b) (5)



Memorandum of Telephone Conversation

Date: June 16, 2010

Between:

Ashley Roberts, Ph.D.

Cantox Health Sciences International
(905) 542-2900

Sharon Choi, Ph.D.

Cantox Health Sciences International

And

Jeremy Mihalov, M.S.

HFS-255

Paulette Gaynor, Ph.D.

HFS-255

Ronald Chanderbhan, Ph.D.

HFS-255

Sylvester Mosley, Ph.D.

HFS-255

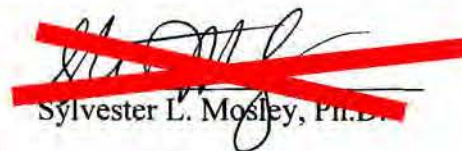
Subject: Status of GRN000324 (Heat-killed *Lactobacillus plantarum*)

On June 16, 2010 at a pre-arranged time, FDA called Cantox, who conducted the call on behalf of the notifier (House Wellness Foods Corporation). Following introductions, FDA personnel opened by stating that we have reviewed the electronic mail correspondences dated May 26, 2010, May 26, 2010, and May 27, 2010 that you submitted on behalf of the notifier as well as a letter dated May 26, 2010 from the notifier.

FDA personnel explained there were still issues surrounding exposure estimates and immunological effects. FDA personnel explained that the gap between the notice's 90th percentile estimated daily intake for the heat-killed *L. plantarum* and the amount investigated in the study that the notifier considers to be pivotal (Hirose 2006) is too great. FDA personnel also explained that the immunological effects mentioned in the notice, including the correspondences noted above, need be discussed in more detail by the notifier, showing how these effects would not be adverse in the short or long term. As a possible way of overcoming these issues, FDA personnel recommended that the notifier look at the long history of use of heat-killed *L. plantarum* in the Japanese population. From this, the notifier could possibly be able to get an estimated daily intake that would be comparable to what is intended in the notice.

Page 2 – June 16, 2010, conversation about GRN 000324 (Heat-killed *Lactobacillus plantarum*)

Cantox stated they would relay the contents of our teleconference to their client and will let FDA know of their client's decision about the notice.


Sylvester L. Mosley, Ph.D.

(b) (5)





January 10, 2011

Xie Dan
Northeast Pharmaceutical Group Co., Ltd.
No. 37, Zhonggong Bei Street
Tiexi District, Shenyang City, Liaoning Province
110026
P.R. CHINA

Re: GRAS Notice No. GRN 000362


Dear Xie Dan,

The Food and Drug Administration (FDA) has received the notice, dated November 15, 2010, that you 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)). FDA received this notice on November 29, 2010, filed it on December 8, 2010, and designated it as GRN No. 000362.


The subject of the notice is levocarnitine. The notice informs FDA of the view of Northeast Pharmaceutical Group Co., Ltd. that levocarnitine is GRAS, through scientific procedures, for use as an ingredient in several food categories, which do not include meat or poultry products.

In accordance with proposed 21 CFR 170.36(f), a copy of the information in this notice that conforms to the information described in the proposed GRAS exemption claim (proposed 21 CFR 170.36(c)(1)) is available for public review and copying via the FDA home page at <http://www.fda.gov>. To view or obtain an electronic copy of this information, follow the hyperlinks from the "Food" topic to the "Food Ingredients and Packaging" section to the "Generally Recognized as Safe (GRAS)" page where the GRAS Inventory is listed. If you have any questions about the notice, contact me at 301-436-1235.

Sincerely yours,


Richard Bonnette
Division of Biotechnology and
GRAS Notice Review
Center for Food Safety
and Applied Nutrition

(b) (5)



Bonnette, Richard

From: Bonnette, Richard
Sent: Friday, March 11, 2011 2:49 PM
To: 'qa@negpf.com.cn'
Subject: RE: withdraw the notice from review GRN 362

Dear Xie Dan,

Thank you for your note. I have begun the process of withdrawing GRN 362 from review. You will be receiving a letter by standard mail noting that FDA has ceased to evaluate the notification at your request. I'll also send an email version to you when it is signed to avoid the lag time involved with sending mail.

Regarding your question about a standard format, we do not have a standard format or table of contents that we recommend for GRAS notifications. Our website has some general guidelines (<http://www.fda.gov/Food/FoodIngredientsPackaging/GenerallyRecognizedasSafeGRAS/default.htm>) and the inventory of GRAS notices (www.fda.gov/grasnoticeinventory) will have links to notifications that have successfully completed the process that you can review as examples. For general guidelines, there is quite a bit of information in the original GRAS Notification Proposed Rule from 1997. There should be a link to this on the general "GRAS" webpage that I cited above.

The questions raised previously, primarily the lack of publication of key studies and a lack of an adequate exposure assessment, remain the most significant outstanding questions. Other issues noted with the notice were largely typographical and probably don't need a detailed description here.

For publications used to support a GRAS determination, these publications should be widely distributed and representative broadly of the scientific community worldwide. I'm not sure whether or not this is possible with publications based in China, I suspect it would depend on the publication.

I also suggest you consider using a consulting service to aid the preparation of your next submission. Many large companies opt to use consultants to help in the preparation and formatting of GRAS submissions. In fact, it is probably more common to receive GRAS submissions from consultants than directly from manufacturers. I cannot recommend any specific consultants, but you can see their names and the names of their companies listed as the contact in many of our response letters in the GRAS inventory (link above).

We agree that a teleconference would be a good way to talk about these issues in more detail and I would be happy to schedule a time convenient for everyone.

With best regards,

Richard Bonnette

From: qa@negpf.com.cn [mailto:qa@negpf.com.cn]
Sent: Tuesday, March 08, 2011 8:50 PM
To: Bonnette, Richard
Subject: withdraw the notice from review GRN 362

Dear Richard Bonnette,

We wish to withdraw the present notice from review regarding GRN 362, levocarnitine. After we supplement it, we will resubmit it.

Before we supplement it, we want to know

1. Do you have the specific table of contents or format for GRAS notice for reference? Does it enough except two significant issues you mentioned in last e-mail?
2. Does FDA have other requirements about the notice?
3. You mentioned in last e-mail that "One possible remedy for this issue would be the publication of the unpublished studies in the peer-reviewed scientific literature. " I don't understand what the peer-reviewed scientific literature is. Do you mean we could publish the unpublished data in China scientific literature or only in American literature?

please advise us.

After we understand the above doubts, we want a teleconference to discuss these items further with FDA. Maybe we will contact some local agent to join the teleconference to facilitate the communication. If you are convenient, can you arrange the teleconference for us in four or five weeks?

Thank you very much for your kind reply.

Xie Dan

Director of QA of NEPG from China

----- 原始邮件 -----
 >From: "Bonnette, Richard" <Richard.Bonnette@fda.hhs.gov>
 >Reply-To:
 >To: "qa@negpf.com.cn" <qa@negpf.com.cn>
 >Subject: Your GRAS notification for levocarnitine (GRN 362) - response to your questions
 >Date: Wed, 2 Mar 2011 13:26:29 -0500
 >

Dear Xie Dan,

Thank you for your prompt reply. When more minor clarifications or additional information is needed during review of a GRAS notice, we typically allow notifiers up to 10 business days for the preparation of a response. However, this only applies to cases where the issues noted are readily correctable by material that can be provided within a few days; generally applying to minor clarifications rather than major issues. For GRN 352, the issues noted are much more significant and, we expect them to be more time consuming to remedy, especially the issue of the safety studies remaining unpublished. In addition to the studies being published, I should also mention that FDA prefers that the studies be published and available in the scientific literature for a "reasonable amount of time" (at least several months) before relying on them to make a GRAS conclusion. This time period provides some evidence that there is agreement within the scientific community about the conclusions of the study that a substance is safe.

With regard to your question about the GRN number scheme, when a notice is withdrawn and a new notice on the subject submitted, a new GRN identifier will be assigned once the revised notice is submitted. Our response letter and website will reference the original GRN submission. An example from previous submissions would be GRN 101 (withdrawn and successfully resubmitted as GRN 127, available here: <http://www.fda.gov/grasnoticeinventory>). After, selecting (clicking the mouse on) the GRN number of the original notice, the new notice number is listed in the field named "additional information." I'm sure there are other examples as well, but this is one that I remembered. I hope this is helpful to you. If you have further questions, please don't hesitate to ask.

With best regards,

Richard Bonnette

From: qa@negpf.com.cn [mailto:qa@negpf.com.cn]
Sent: Tuesday, March 01, 2011 11:37 PM
To: Bonnette, Richard
Subject: Re: Your GRAS notification for levocarnitine (GRN 362)

Dear Richard Bonnette,

I have received your email about the assessment of our GRAS notice (GRN 362) of levocarnitine. We will consider the significant issues found by the review team and re-evaluate our notice file. Maybe some modification is necessary.

But I have not decided to withdraw it or not. Before we make the decision, I want to know the following issues, please provide some advice to me:

1. If we don't withdraw this notice, how long would you allow us to provide the additional information to remedy the notice?
2. If we withdraw this notice, is the GRAS number 362 kept for us? When we resubmit the notice, do you assign another number for us?

After evaluation of the GRAS notice combining your findings and your reply to the above questions, we will tell you our decision.

Best regards,

Xie Dan

QA Director of NEPG from China

----- 原始邮件 -----
 >From: "Bonnette, Richard" <Richard.Bonnette@fda.hhs.gov>

>Reply-To:
 >To: "qa@negpf.com.cn" <qa@negpf.com.cn>
 >Subject: Your GRAS notification for levocarnitine (GRN 362)
 >Date: Tue, 1 Mar 2011 09:24:35 -0500
 >

Dear Xie Dan,

This email is in regards to the U.S. Food and Drug Administration's (FDA) review of your GRAS notification, dated November 15, 2010, for levocarnitine (GRN 362). This email will describe several significant issues with your submission identified by FDA reviewers. You will need to submit additional information to remedy these issues. In other cases where substantial issues have been identified during the review of GRAS notices, we have encouraged notifiers to inform us of their intention to withdraw the notice from review. There is no penalty for withdrawing the notice, and it is a frequently-used mechanism to provide additional time for a notifier to remedy issues identified during FDA's review. Given the substantial nature of the issues described below, we advise you to withdraw the present notice (GRN 362) for levocarnitine and to resubmit the notice to FDA once you have information to address these issues. Please note that this letter is intended to communicate only the most significant issues identified. There are other, less significant, issues and points of clarification that will need to be addressed as well in any future submission.

The significant issues noted by the review team relate primarily to two areas. The first issue is described in Section 170.30(b) of Title 21 of the U.S. Code of Federal Regulations (21 CFR), outlining the criteria for determining the eligibility of a use of a substance in food as "Generally Recognized as Safe (GRAS)." This regulation states that the "general recognition" component of GRAS shall ordinarily be based upon published studies. The publication of studies is a mechanism for demonstrating that there is a general recognition of safety of the use of the substance in food.

In the present case, the review team noted that the animal toxicity studies described by the notice were unpublished. While we do note that two clinical studies and an *in vitro* study were published, the review team finds that these published studies are not sufficient to demonstrate the safety of exposure to levocarnitine resulting from the proposed uses of levocarnitine in food. In particular, the review team found that the clinical studies were not designed to identify potential adverse effects and were of limited applicability to the general population since they were conducted in specific subpopulations, including cancer patients. While FDA does consider unpublished studies in its review of GRAS notifications, unpublished studies can only be considered to be supportive or corroborative of other published studies or other evidence of general recognition of safety. One possible remedy for this issue would be the publication of the unpublished studies in the peer-reviewed scientific literature.

Based on our review of the GRAS notice, the second significant issue noted is that the review team has a limited understanding of the intended uses (use levels in food categories) described in your notice and the likely dietary exposures to levocarnitine that would result in the U.S. population from these uses. Typically, the proposed concentrations (or amounts per serving) of a food ingredient in specific food categories are used to calculate a probable dietary exposure in the general population – an estimated daily intake or EDI. Estimates of exposure are then compared to the highest levels that have no (adverse) effects in toxicity studies, which would provide evidence that those exposures from the proposed uses would not present a safety issue. I have provided a link here: <http://www.fda.gov/food/guidancecomplianceregulatoryinformation/guidancedocuments/foodingredientsandpackaging/ucm074725.htm>) to FDA guidance describing suggestions for how an exposure assessment might be prepared.

Your notice on page 2 identifies several food categories and proposed levels of use, including a use that was unclear to us. You cite "formula milk powder for children" as a potential intended use. It wasn't clear if you intend this to be infant formula as defined by U.S. law in Section 201(z) of the Federal Food, Drug and Cosmetic Act. This section defines infant formula as, "a food which purports to be or is represented for special dietary use solely as a food for infants by reason of its simulation of human milk or its suitability as a complete or partial substitute for human milk." If you do intend your ingredient to be used in infant formula, please consult with us to discuss the additional regulatory requirements and material that will be needed to support the safety of this intended use, as infants are a vulnerable population and its use as the major component in a diet must be considered. Page 2 also cites "sports nutrition food," which lists "1-4g/day." The cited "1-4 grams per day" seems to be in terms of human exposure, rather than level of use (that is, usually these levels would be expressed in amounts (grams) per serving. Further, it isn't clear from the description which types of foods would be considered "sports nutrition foods."

If you have additional questions about these issues, it may be possible for us to arrange a teleconference to discuss these items further. We would also be willing to schedule a meeting with you, or any local agent you designate, to discuss your submission. If you wish to withdraw the present notice from review, please inform us by letter, FAX (301-436-2965) or to my email address (Richard.Bonnette@fda.hhs.gov) noting that you wish to withdraw the notice from review. Please acknowledge receipt of this email by replying when you receive it.

With best regards,

Richard Bonnette

Richard Bonnette, M.S.
 Consumer Safety Officer
 U.S. Food and Drug Administration
 Center for Food Safety and Applied Nutrition

Office of Food Additive Safety
Phone: 301-436-1235
FAX: 301-436-2965

Robert Baldo, Gillian L

From: Mark Itzkoff [mitzkoff@ofwlaw.com]
Sent: Monday, August 15, 2011 12:30 PM
To: Robert Baldo, Gillian L
Cc: Merker, Robert I; Dongen, Ton van; 'Ashley Roberts Intertek'
Subject: RE: GRN 378
Attachments: Reply GRN 378 Confidential.mli.pdf; Reply GRN 378 public.mli.pdf; P 33 revised.pdf

Dr. Robert-Baldo,

On behalf of PURAC, we are hereby submitting the attached response to your request for additional information on the GRAS Notice for Food Ferment Solutions, GRN 378. Attached are the following: (1) the confidential response to your request; (2) a reacted copy of the confidential response; and (3) an amended page 33 to the original Notice.

If you have any additional questions regarding the Notice, please do not hesitate to contact us.

Regards,

Mark Itzkoff

Mark L. Itzkoff
OFW Law
202 518-6327
www.ofwlaw.com

New address for OFW Law effective July 1, 2011:

600 New Hampshire Ave. NW, Suite 500
Washington, DC 20037
(No change to phone or email)

The preceding e-mail message contains information that is confidential, may be protected by the attorney/client or other privileges, and may constitute non-public information. This message is intended to be conveyed only to the designated recipient (s). If you are not an intended recipient of this message, please notify the sender immediately at (202) 518-6327. Unauthorized use, dissemination, distribution, or reproduction of this message is strictly prohibited and may be unlawful.

From: Robert Baldo, Gillian L [mailto:Gillian.RobertBaldo@fda.hhs.gov]
Sent: Wednesday, August 03, 2011 11:40 AM
To: Mark Itzkoff
Cc: Merker, Robert I
Subject: GRN 378

Dear Mr. Itzkoff:

We are resending the email below as we have not heard from you with regard to our request for clarification about the substrate nor the issues that we noted during the telephone conference held on June 30, 2011. At that time we indicated that we expected a timely response. We understand that CANTOX did speak to Dr. Dinovi on July 20, 2011 about the exposure calculations.

We suggest that you withdraw the notification if you cannot provide the requested clarifications by August 15, 2011.

We look forward to hearing from you soon.

Sincerely,

Gillian Robert-Baldo PhD
On Detail
Consumer Safety Officer
Division of Biotechnology
and GRAS Notice Review
OFAS/CFSAN/FDA
240-402-1016 (desk)
240-402-1460 (voice)

From: Robert Baldo, Gillian L
Sent: Monday, July 11, 2011 3:07 PM
To: 'Mark Itzkoff'
Cc: Merker, Robert I
Subject: Clarification requested regarding substrates for fermentation - GRN 378

Dear Mr. Itzkoff:

In addition to the points raised during our telephone conference of June 30, 2011, we would appreciate the following clarification concerning GRN 000378:

On page 5 under the heading *Common Name of the Notified Substance* and on page 14 under the heading *Product identity* the list of *natural substrates* for fermentation includes: *dairy sources, sugars, wheat, malt and fruit- and vegetable- based sources*. However, on page 15 under the heading of *Method of Manufacture* the following substances are listed as fermentation substrates: *caramel, dairy sources (lactose, whey, and whey permeate, milk, milk solids, yogurt), fruit- and vegetable-based sources (including juices, pastes and peels), honey, maple syrup, molasses, starch (from barley, corn, malt, potato, rice, tapioca, and wheat), sugars (from corn, beet, palm or sugar cane), and wheat*. On page 30 under the summary, the following are listed as fermentation substrates: *dairy sources (milk, milk solids, whey powder, lactose), sugars (from corn, beet or sugar cane), wheat, malt, and fruit- and vegetable-based sources (including juices, pastes and peels)*.

Please clarify what you intend to list as your fermentation substrates; your lists are inconsistent and there are disparities between the broader categories and specific substrates. As some examples: you have included caramel, honey, molasses and maple syrup, but the category they would fall in isn't clear, and you have listed wheat and malt in two of your lists, but in another case you have listed starch (from barley, corn, malt, potato, rice, tapioca and wheat) and wheat.

If you have any questions, please contact us. Thank you.

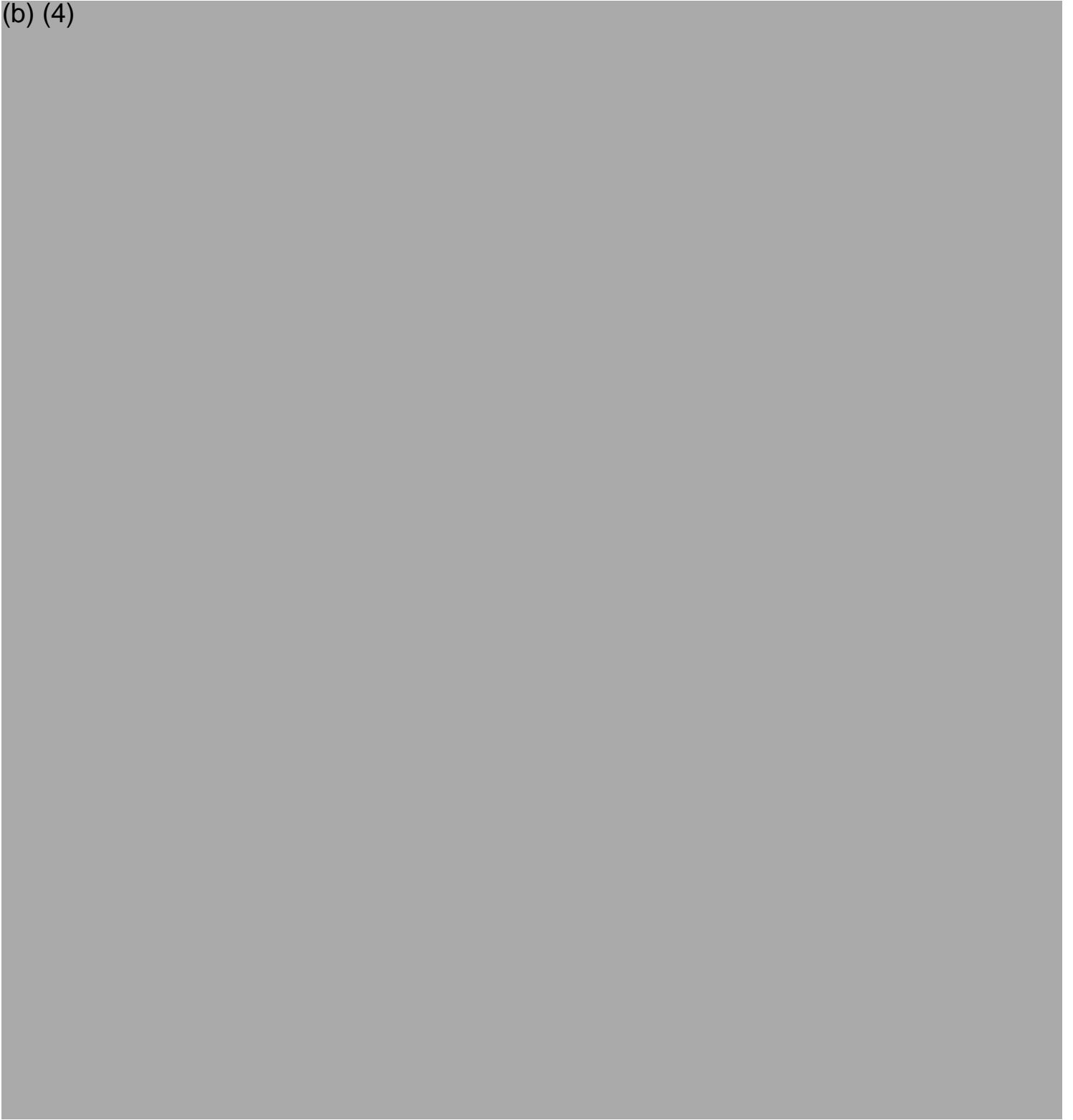
Sincerely,

Gillian Robert-Baldo PhD
On Detail
Consumer Safety Officer
Division of Biotechnology
and GRAS Notice Review
OFAS/CFSAN/FDA
240-402-1016 (desk phone)
24-402-1416 (voice mail)



CONFIDENTIAL


(b) (4)





CONFIDENTIAL


(b) (4)





CONFIDENTIAL

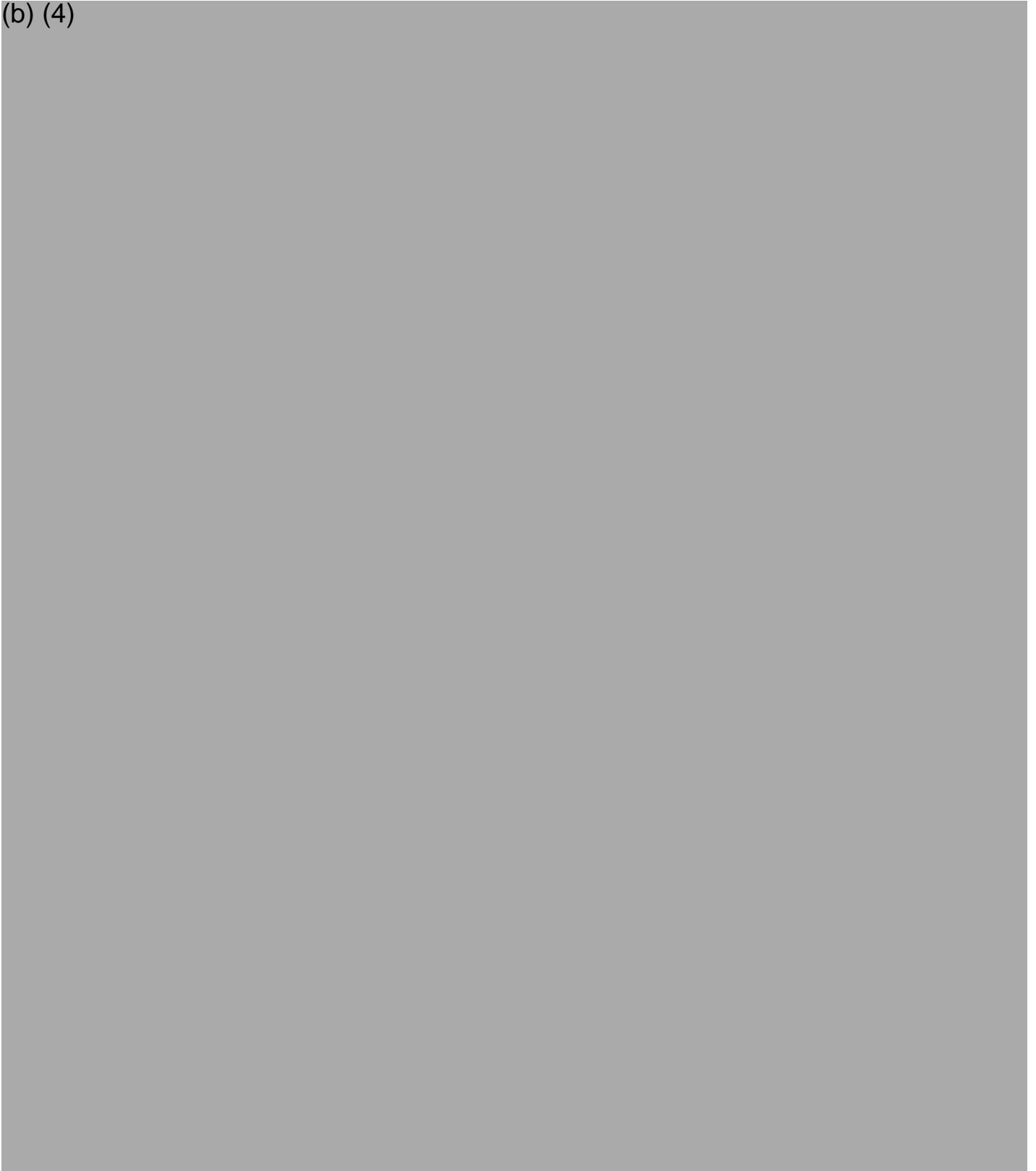
(b) (4)





CONFIDENTIAL

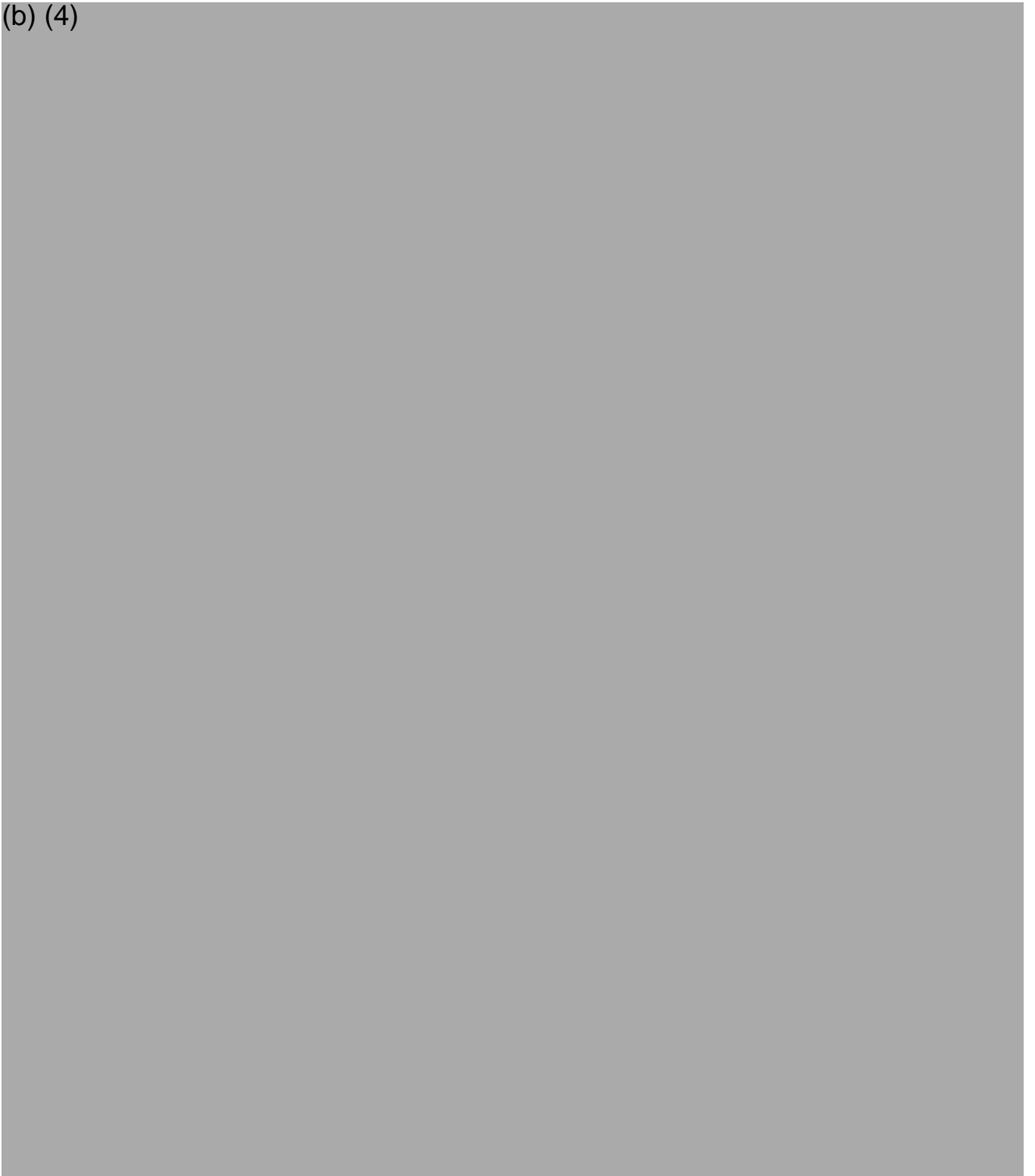
(b) (4)





CONFIDENTIAL


(b) (4)





CONFIDENTIAL

(b) (4)





To : OFW – Mark Itzkoff, CANTOX – Ashley Roberts
From : Purac
Your ref : Ton van Dongen

July 20, 2011

Re : **Additional information submission GRN378**

The purpose of this memo is to provide additional information to support the GRAS submission GRN378. This information being:

- Organisms GRAS submission Food Ferment Solutions
- Clarification substrate sources definition page 5, 14, 15, 30
- Correction reference page 33

Organisms GRAS submission Food Ferment Solutions

FDA Q: In fermentation process is a single organism strain used for each product or are multiple strains used? Are the microorganisms used “deposited”?

In the table below, an overview of the species is given including their deposit number. Not all species are deposited.

Name	Deposition number ¹	EFSA QPS granted	source
<i>Streptococcus thermophilus</i>		Yes	
<i>Bacillus coagulans</i>		Yes	
<i>Lactobacillus acidophilus</i>		Yes	
<i>Lactobacillus paracasei</i> subsp <i>paracasei</i>		Yes	
<i>Lactobacillus plantarum</i>		Yes	
<i>Lactobacillus sakei</i>		Yes	
<i>Lactobacillus bulgaricus</i>		Yes ²	
<i>Propionibacterium freudenreichii</i> subsp. <i>shermanii</i>		Yes	

¹ ATCC is a number from the American Culture Collection, DSM is a number from the German culture collection

² *L. bulgaricus* is now considered to be a subspecies of *L. delbrueckii* (Weiss et al 1983)



Some of the ferments are produced by fermentation by one species whereas others are produced by a mixed culture. Also multiple strains from the same species are used in one culture.

Example of fermentation with a mixed culture: milk is fermented with a mixed culture of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. Both can acidify milk, but together they show a much higher activity. Optionally the product can be further fermented with *Propionibacterium* or *Propionibacterium* can be added simultaneously with the lactic acid bacteria, enabling a simultaneous fermentation.

Clarification substrate sources definition

FDA Q (E-Mail Gillian Robert-Baldo PhD): Please clarify what you intend to list as your fermentation substrates.

The fermentation substrates we intend to list for all pages 5, 14, 15 and 30 is consistently: dairy sources (including milk, milk solids, whey, whey powder, whey permeate, lactose, yogurt), sugars (including those from corn, beet, palm or sugar cane) and sugar sources (including honey, maple syrup, molasses, caramel) starches (including those from barley, corn, potato, rice, wheat, malt and tapioca), fruit- and vegetable-based sources (including juices, pastes and peels).

Correction reference

FDA Q: Correction of reference to FASEB (1978) on page 33 of the submission.

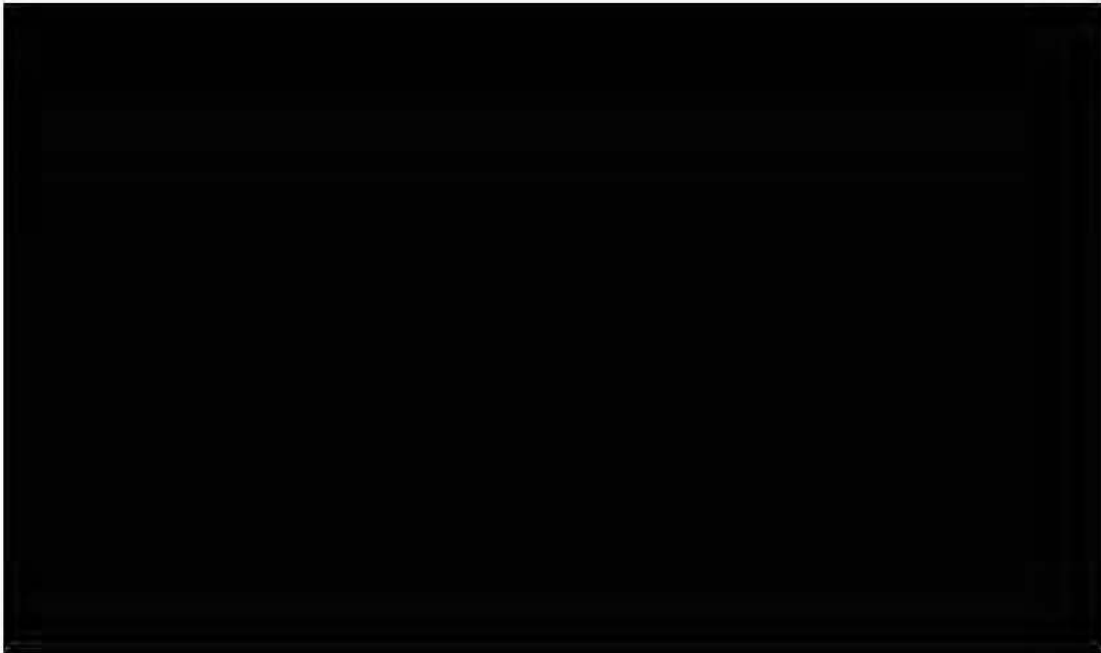
The correct reference on page 33 should be:

FASEB (1978). Evaluation of the Health Aspects of Lactic Acid and Calcium Lactate as Food Ingredients. (FDA/BF-78/108; PB238-713; SCOGS-116). Prepared by Bethesda (MD):Federation of American Societies for Experimental Biology (FASEB), Life Sciences Research Office (LSRO) for Washington (DC):U.S. Food and Drug Administration (U.S. FDA), Bureau of Food.

An amended page 33 is attached.



FDA Q: provide an explanation as to why the dietary estimates are 5- or 10- fold times the intakes that would be found if the actual ferment intakes were measured.



- EC (2008). Regulation (EC) No 299/2008 of the European Parliament and of the Council of 11 March 2008 amending Regulation (EC) No 396/2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin, as regards the implementing powers conferred on the Commission (1) OJ C 161, 13.7.2007, p. 45. 2008. Regulation (EC) No 178/2006 (OJ L 29, 2.2.2006, p. 3). Off J Eur Union 53(L97):67-71. Available at: <http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2008:097:0067:0071:en:PDF>.
- Ennahar S, Aoude-Werner D, Sorokine O, Van Dorsselaer A, Bringel F, Hubert JC et al. (1996). Production of pediocin AcH by *Lactobacillus plantarum* WHE 92 isolated from cheese. Appl Environ Microbiol 62(12):4381-4387.
- Essid I, Medini M, Hassouna M (2009) Technological and safety properties of *Lactobacillus plantarum* strains isolated from a Tunisian traditional salted meat. Meat Sci 81(1): 203-208.
- FAO/WHO (2002). *Human Vitamin and Mineral Requirements*. Report of a Joint FAO/WHO Expert Consultation, Bangkok, Thailand. Rome, Italy: Food and Agricultural Organization of the United Nations (FAO) / World Health Organization (WHO). Available at: <http://www.fao.org/docrep/004/y2809e/y2809e00.htm>.
- FASEB (1978). *Evaluation of the Health Aspects of Lactic Acid and Calcium Lactate as Food Ingredients*. (FDA/BF-78/108; PB238-713; SCOGS-116). Prepared by Bethesda (MD):Federation of American Societies for Experimental Biology (FASEB), Life Sciences Research Office (LSRO) for Washington (DC):U.S. Food and Drug Administration (U.S. FDA), Bureau of Food.
- Gibala MJ, MacLean DA, Graham TE, Saltin B (1998). Tricarboxylic acid cycle intermediate pool size and estimated cycle flux in human muscle during exercise. Am J Physiol 275(2 Part 1):E235-E242.
- Hickson M, D'Souza AL, Rajkumar C, Bulpitt CJ, Muthu N, Rogers TR et al. (2007). Use of probiotic *Lactobacillus* preparation to prevent diarrhoea associated with antibiotics: randomised double blind placebo controlled trial. Br Med J 335(7610):80-83.
- Holzapfel WH, Haberer P, Geisen R, Bjorkroth J, Schillinger U (2001). Taxonomy and important features of probiotic microorganisms in food and nutrition. Am J Clin Nutr 73(2, Suppl.):365S-373S.
- Hüfner E, Markieton T, Chaillou S, Crutz-Le Coq AM, Zagorec M, Hertel C (2007). Identification of *Lactobacillus sakei* genes induced during meat fermentation and their role in survival and growth. Appl Environ Microbiol 73(8):2522-2531.
- IOM (1997). *Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride*. National Academy of Sciences, Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board, Institute of Medicine (IOM). Washington (DC):National Academy Press (NAP). Available at: <http://www.nap.edu/books/0309063507/html/index.html>.

Robert Baldo, Gillian L

From: West-Barnette, Shayla
Sent: Tuesday, September 20, 2011 9:31 AM
To: Robert Baldo, Gillian L
Subject: FW: GRN 378
Attachments: Sept 19 letter.pdf; Revised Intake Section Sept 19'11-mli.pdf

Hi Gillian,

I received an email with attachments from Mark Itzkoff (agent for PURAC) regarding GRN 378. The email is below and the attachments are included with this message. I spoke with Susan Carlson this morning and was advised that you are still working on GRN 378. Can you please forward the information from Mr. Itzkoff to the review team?

Also, if I receive the hard copies that Mr. Itzkoff refers to in his email, I will be sure to give them to you.

Thanks,

Shayla

From: Mark Itzkoff [mailto:mitzkoff@ofwlaw.com]
Sent: Monday, September 19, 2011 4:20 PM
To: West-Barnette, Shayla
Cc: Emily Poly
Subject: GRN 378

Dr. West-Barnette

Attached is the amendment to GRN 378 along with a cover letter for this submission.

We will be sending you 3 copies of the attachment via Federal Express.

If you have any questions, please let me know.

Regards,

Mark Itzkoff

Mark L. Itzkoff
OFW Law
202 518-6327
www.ofwlaw.com

(No change to phone or email)


The preceding e-mail message contains information that is confidential, may be protected by the attorney/client or other privileges, and may constitute non-public information. This message is intended to be conveyed only to the designated recipient(s). If you are not an intended recipient of this message, please notify Olsson Frank Weeda Terman Matz PC immediately at (202) 518-6327. Unauthorized use, dissemination, distribution, or reproduction of this message is strictly prohibited and may be unlawful.

FD

**Ramos-Valle, Moraima**


From: Ramos-Valle, Moraima
Sent: Wednesday, May 04, 2011 12:08 PM
To: Farias, Bianca
Subject: RE: food fermented solutions GRAS submission

(b) (5)



From: Ramos-Valle, Moraima
Sent: Friday, April 29, 2011 3:10 PM
To: Farias, Bianca
Cc: Carlson, Susan
Subject: food fermented solutions GRAS submission

(b) (5)



000061



May 16, 2011

Ton van Dongen
Purac
Arkelsedjik 46
4206 AC Gorinchem
The NETHERLANDS

Re: GRAS Notice No. GRN 000378

Dear Mr. van Dongen


The Food and Drug Administration (FDA) has received the notice, dated April 12, 2011, that you 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)). FDA received this notice on May 3, 2011, filed it on May 3, 2011, and designated it as GRN No. 000378.

The subject of the notice is what you have referred to as "fermented food solutions". The notice informs FDA of the view of Purac that "fermented food solutions" are GRAS, through scientific procedures, for use as antimicrobials in selected food categories, including baked goods and baking mixes, beverages and beverage bases (carbonated and non-carbonated), breakfast cereals, cheeses, coffee and tea, condiments and relishes, fats and oils, fish products (excluding catfish), frozen dairy desserts and mixes, gelatins, puddings and fillings, grain products and pastas, gravies and sauces, meat products, milk products, plant protein products poultry products, processed fruits and fruit juices, processed vegetables and vegetable juices, soups and soup mixes, and sweet sauces, toppings and syrups at levels of 0.1 to 4.5 %. You have stated that "Ferment Food Solutions" are not intended for use in infant formula or infant foods.

A Memorandum of Understanding between FDA and the United States Department of Agriculture (USDA) provides for the review of food ingredients used in the production of meat and poultry products. FDA will send a copy of GRN No. 000378 to the Risk, Innovations, and Management Division of the Food Safety Inspection Service of USDA for their review.

In accordance with proposed 21 CFR 170.36(f), a copy of the text of this letter responding to GRN 000378, as well as a copy of the information in this notice that conforms to the information in the GRAS exemption claim (proposed 21 CFR 170.36(c)(1)), is available for public review and copying at <http://www.fda.gov/grasnoticeinventory>. If you have any questions about the notice, contact me at gillian.robertbaldo@fda.hhs.gov or telephone 240-402-1016 or 240-402-1460.

Sincerely yours,


Gillian Robert-Baldo PhD
Division of Biotechnology and
GRAS Notice Review
Office of Food Additive Safety
Center for Food Safety
and Applied Nutrition



DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration

Memorandum

Date May 16, 2011

From Consumer Safety Officer, Office of Food Additive Safety (HFS-255), Center for Food Safety and Applied Nutrition (CFSAN), Food and Drug Administration (FDA), 5100 Paint Branch Parkway, College Park, MD 20740.


Subject GRN 000378; Use of "Food Ferment Solutions"¹ as an antimicrobial.

To William K. Shaw, PhD, Director, Risk, Innovations, and Management Division, Office of Policy and Program Development, Food Safety and Inspection Service (FSIS), George Washington Carver Center (GWCC), 5601 Sunnyside Avenue, Mail STOP 5271, Beltsville, MD 20705-5271.

As per the Memorandum of Understanding between FDA/CFSAN and FSIS/LPDD, I am requesting consultation on GRAS Notice No. GRN 000378 for the use of "Food Ferment Solutions." Purac has determined that "Food Ferment Solutions" are GRAS, through scientific procedures, for use as an antimicrobial in selected food categories including baked goods and baking mixes, beverages and beverage bases (carbonated and non-carbonated), breakfast cereals, cheeses, coffee and tea, condiments and relishes, fats and oils, fish products (excluding catfish), frozen dairy desserts and mixes, gelatins, puddings and fillings, grain products and pastas, gravies and sauces, meat products, milk products, plant protein products poultry products, processed fruits and fruit juices, processed vegetables and vegetable juices, soups and soup mixes, and sweet sauces, toppings and syrups at a levels of 0.1 to 4.5 %. Specifically, I am requesting that FSIS provide advice to FDA, in writing, on any criteria, restrictions, conditions of use, or prohibitions that FSIS believes necessary concerning use of the substance in products subject to the Federal Meat Inspection Act or the Poultry Products Inspection Act.

Please direct your written response to my attention. If you have any questions, I can be reached by telephone at (240) 402-1016 or (240) 402-1460, by telefax at (301) 436-2965, or by electronic mail at gillian.robertbaldo@fda.hhs.gov.

Thank you.


Gillian Robert-Baldo, PhD

Enclosure:

¹FDA will likely refer to this by a different descriptive name in its letter.

MEMORANDUM OF TELEPHONE CONFERENCE

Date: June 30, 2011

Between:

Mark Itzoff
Maureen de Wispelaere
Ton van Dongen
Edwin Bontenbal
Willem Hommes

Olsson Frank Weeda Terman Matz PC
PURAC
PURAC
PURAC
PURAC

And

Susan Carlson
Ronald Chanderbhan
Michael DiNovi
Robert Merker
Aydin Orstan
Gillian Robert-Baldo
Shayla West-Barnette

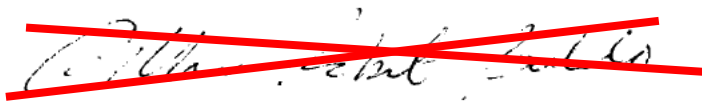
HFS-255
HFS-255
HFS-255
HFS-255
HFS-255
HFS-255 (on detail)
HFS-255

Subject: Discussion with PURAC on the Deficiencies for GRN 000378

We arranged a meeting with PURAC and Mr. Itzkoff, its representative, to discuss some issues regarding the bacteria used for fermentation and exposure calculations that were raised during our 30 day meeting. We asked the following questions about the microorganisms:

- 1) Please clarify whether the fermentation microorganisms described in the notice include several strains of the same species. Also, please provide the strain designations of the fermentation microorganisms.
- 2) Please discuss whether the strains used as fermentation microorganisms are maintained in a recognized culture repository.

We noted that the exposure estimation calculations did not represent a realistic approach as they were based on maximum usage and did not consider replacement use; part of the problem was that exact formulations have not yet been established, but will be determined by PURAC's customers. The CANTOX consultant involved with these calculations was not available for the phone call. The CANTOX consultant will contact Dr. Michael Di Novi with questions. PURAC was told that we expect a timely response and it indicated that its responses would be supplied in writing.



Gillian Robert-Baldo, PhD

(b) (5)

MEMORANDUM OF TELEPHONE CONFERENCE

Date: August 24, 2011

Between:

Mark Itzoff
Ton van Dongen
Edwin Bontenbal
Willem Hommes
Ashley Roberts

Olsson Frank Weeda Terman Matz PC
PURAC
PURAC
PURAC
CanTox

And

Michael DiNovi
Gillian Robert-Baldo

HFS-255
HFS-255 (on detail)

Subject: Discussion with PURAC on Exposure Calculations dated August 15, 2011 for GRN 000378

We arranged a teleconference with PURAC and Mr. Itzkoff, its representative, to discuss Purac's August 15, 2011 response to our question on exposure calculations, raised during our 30 day meeting.

The following is a summary of our conversation with PURAC and its representatives:

We stated that PURAC had supplied two copies of its response, one being redacted for public release, and noted that exposure estimates are part of the safety assessment which cannot be confidential.

We explained that the response on the exposure estimates did not resolve the problem with the worst case exposure levels being of toxicological concern, noting potassium, calcium, lactate and propionate in particular. We stated that the maximum use levels in the Table 1.D.2.1 are unrealistic and problematic. We outlined three options open to PURAC; 1) leave things as they are and receive a "bad day" letter; 2) submit an amendment to the notice that replaces the current exposure estimates and includes exposure calculations with replacement-use considerations and any changes in background levels for four potential or proposed products, with attention to potassium, calcium, lactate and propionate instead of the current table, or 3) withdraw GRN 000378 and resubmit a new GRN with change as described in no. 2. PURAC was concerned about timing and stated it would probably do an amendment. It then wondered if the worst case exposures were discussed in other parts of the notification. We left it to PURAC to decide what it needed to do. PURAC inquired whether the use of specific examples might hinder future development of products. We indicated that this would not be a problem.



Gillian Robert-Baldo, PhD



Memorandum

Date	October 17, 2012
From	Aydin Örstan (HFS-255) Through Edwin Flamm (HFS-255) Gillian Robert-Baldo (HFS-255)
Subject	GRN 000378
To	Administrative File GRN 000378

Keywords: sodium, potassium, lactate, bacterial fermentation

This memorandum summarizes the notice dated April 13, 2011, that Mark L. Itzkoff submitted on behalf of Purac, 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 GRAS proposal). FDA received this notice on May 3, 2011, filed it on May 3, 2011, and designated it as GRN No. 000378.

The subjects of the notice are cultured dairy sources, sugars, wheat, malt, and fruit- and vegetable-based sources fermented by *Streptococcus thermophilus*, *Bacillus coagulans*, *Lactobacillus acidophilus*, *Lactobacillus paracasei* subsp. *paracasei*, *Lactobacillus plantarum*, *Lactobacillus sakei*, *Lactobacillus bulgaricus* and *Propionibacterium freudenreichii* subsp. *shermanii* or mixtures of these microorganisms. This memorandum will refer to the subjects of the notice as "cultured substrates". The notice informs FDA of the view of Purac that cultured substrates are GRAS, through scientific procedures, for use as antimicrobial agents in a variety of food categories.

This memorandum summarizes the data and information the notifier describes to support its view that the intended use of cultured substrates is GRAS.

Identity, Manufacturing, Composition and Specifications

The subjects of the notice are cultured substrates fermented by *Streptococcus thermophilus*, *Bacillus coagulans*, *Lactobacillus acidophilus*, *Lactobacillus paracasei* subsp. *paracasei*, *Lactobacillus plantarum*, *Lactobacillus sakei*, *Lactobacillus bulgaricus*, and/or *Propionibacterium freudenreichii* subsp. *Shermanii*, or mixtures of these microorganisms. In an amendment dated August 15, 2011, Purac listed the fermentation substrates they intend to use as: dairy sources (including milk, milk solids, whey, whey powder, whey permeate, lactose, yogurt), sugars (including those from corn, beet, palm or sugar cane) and sugar sources (including honey, maple syrup, molasses, caramel) starches (including those from barley, corn, potato, rice, wheat, malt and tapioca), and fruit- and vegetable-based sources (including juices, pastes and peels).

Following fermentation, a base is added to neutralize the liquid. The fermentation end product is then purified by removing the biomass and other impurities, followed by concentration and spray drying. Table II.B-1 (p. 17) in the notice lists the processing aids used in the manufacture of cultured substrates.

The final product is a mixture of sodium, potassium and calcium salts of lactic acid, acetic acid and propionic acid, as well as sugars (glucose, fructose, sucrose, lactose and galactose), protein and succinic acid. Tables II.C.1-1 and II.C.1-2 in the notice list Purac's specifications for cultured substrates. These include specifications for individual ions, protein, sugars, succinic acid, lead and microbial limits. Purac also provided the results of analyses of three batches of its product in powder form and three batches in liquid form (Tables II.C.1-3 through II.C.1-6). All of the analyzed batches complied with the specifications.

Intended Conditions of Use and the Estimated Daily Intake Levels

The notifier intends to use cultured substrates in the U.S. in numerous food categories, including baked goods and baking mixtures, beverages and beverage bases (carbonated and non-carbonated), breakfast cereals, cheeses, coffee and tea, condiments and relishes, fats and oils, fish products (excluding catfish), frozen dairy desserts and mixes, gelatins, puddings and fillings, grain products and pastas, gravies and sauces, meat products, milk products, plant protein products, poultry products, processed fruits and fruit juices, processed vegetables and vegetables juices, soups and soup mixes, sweet sauces, toppings and syrups. The intended uses exclude infant formula and infant foods. According to the notice, cultured substrates will be added to foods typically at levels of 0.1 to 4.5%. The notice also states that use levels will not exceed 0.16% for sodium and calcium, 0.75% for potassium, 2.1% for lactate, 0.6% for acetate and propionate, 0.9% for protein, 0.25% for sugar and 0.1% for succinic acid.

The notice explains that the product composition of cultured substrates, and consequently, the levels of individual components within the product, will vary depending on the starting materials used, the conditions of the fermentation process and specific customer needs. To account for the variability of the end product composition, in the original notice, Purac determined intakes using the maximum use levels for each component in cultured substrates. Purac estimated the 90th percentile all-user intakes for all proposed food uses of sodium, calcium, potassium, lactate, acetate, propionate, protein, sugar and succinic acid as 2.3, 2.3, 15.8, 30.2, 12.7, 12.7, 12.9, 5.3 and 2.1 g/person/day, respectively (45, 45, 281, 590, 225, 225, 253, 94 and 37 mg/kg body weight/day, respectively).

In an amendment dated September 19, 2011, Purac noted that the original intake estimates noted above were gross overestimates of the actual intakes and provided intake estimates that more accurately reflect the actual consumption of the major components of cultured substrates from the intended uses. These estimates were calculated from intakes of four types of cultured substrates that are intended for use in specific food applications. For example, the product PuraQ RS20 P, which is intended for use in cheese, contains calcium at levels of 11.0 to 14.0%, as calcium is the preferred cation for dairy products, but contains no sodium or potassium. As a result, Purac noted that the actual exposure to the major components of cultured substrates in each example product is far lower than originally estimated using the maximum levels of each component. For example, Purac estimated the 90th percentile all-user intakes for calcium, lactate, acetate and propionate for all

proposed food uses of PuraQ RS20 P as 109, 359, 47 and 94 mg/person/day, respectively (1.7, 5.5, 0.7 and 1.4 mg/kg body weight/day, respectively).

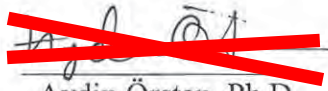
In the original notice and in the September 19 amendment, Purac noted that for several proposed uses, cultured substrates will be used as direct replacement for existing lactate products that are currently in the food supply and, therefore, there will be no net increase in lactate intake resulting from these uses. Since the intake estimates assume that all uses of cultured substrates products will be new uses of lactates, the actual increase in exposure to lactate will be lower than indicated in the exposure estimates.

Safety


PURAC reports on the safety of the cultured substrates and notes that all components of cultured substrates occur naturally in food, are approved for food use in the United States population, and/or are endogenous to the body. PURAC also reports on several published expert organizations and committee papers (Institute of Medicine, Joint Expert Committee on Food Additives) to further support safety. PURAC also discusses the nonpathogenic and nontoxigenic nature of the microorganisms that are used in the production of cultured substrates. PURAC states that these microorganisms do not produce antimicrobials during the manufacturing process for cultured substrates. PURAC also states that these microorganisms are commonly used as food ingredients, as well as in food processing applications.

Conclusions

We have evaluated the information in GRN 000378 and have not identified issues that would contradict the notifier's conclusion that cultured substrates are GRAS for use as a food ingredient.

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Aydin Örstan, Ph.D.

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MEMORANDUM OF MEETING

Date: February 7, 2011

Time: 2:00 pm – 3:00 pm

Location: FDA, Center for Food Safety and Applied Nutrition, Office of Food Additive Safety, 4300 River Road, College Park, MD 20740

Participants:

Visitors:

Mark L. Itzkoff

Ashley Roberts

Andrea Wong

Ton von Dongen (by telephone)

Olsson Frank Weeda Terman Bode
& Matz PC

Cantox Health Sciences International

Cantox Health Sciences International

PURAC

FSIS:

David Zeitz, DVM (by telephone)

FDA/CFSAN:

Shayla West-Barnette, Ph.D.

Ron Chanderbhan, Ph.D.

Edwin Flamm

Robert I. Merker, Ph.D.

Aydin Orstan, Ph.D.

Sylvester Mosley, Ph.D.

Mike DiNovi, Ph.D.

OFAS/DBGNR (HFS-255)

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Subject: Fermentation Products

Cantox presented an overview of PURAC and stated that PURAC has been developing products with natural lactic acid-based chemicals for eighty years. Cantox also discussed the broad application range and uses for PURAC's food products.

Cantox discussed PURAC's fermentation products, which are blends of sodium or potassium organic salts, as well as sugars. Cantox discussed the manufacturing process for their fermentation products, stating that the production microbes have long histories of use in food production or are permitted for use in food production in the U.S. Cantox also discussed the intended uses and use levels for the products. Cantox further discussed the metabolic fate and safety of the individual components of the products.

Cantox stated that FDA had no questions in response to GRN 240, the subject of which was another fermentation product developed by PURAC.

In response to a question from FDA, Cantox stated that there are several product formulations for which PURAC is considering contacting FDA. FDA advised Cantox to provide descriptive names for each product formulation and to include the substrate name in the product name. FDA also advised Cantox to provide total chemical composition intake calculations and information about specific microorganisms used in the production of each product formulation.

After the meeting, visiting participants were given copies of the attendee list, as well as Dr. West-Barnette's contact information.


~~Shayla West-Barnette~~
Shayla West-Barnette, Ph.D.

ATTACHMENTS

- 1) Pre-submission Meeting Request
- 2) Re-submission Meeting Agenda
- 3) Business Cards for Mark Itzkoff and Dr. Andrea Wong

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(b) (5)



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West-Barnette, Shayla

From: Merker, Robert I
Sent: Tuesday, January 11, 2011 10:02 AM
To: West-Barnette, Shayla
Subject: FW: Pre-Submission Conference for PURAC

Shayla, Would you please make arrangements with Mr. Itzkoff for this presubmission request.

I will ask Sharon Dodson to enter the meeting request as correspondence.

From: Mark Itzkoff [mailto:mitzkoff@ofwlaw.com]
Sent: Monday, January 10, 2011 1:18 PM
To: Merker, Robert I
Subject: FW: Pre-Submission Conference for PURAC

Dr. Merker

Attached is the e-mail to Dr. Martin that we discussed a few moments ago.

Thank you for your assistance.

Mark Itzkoff

From: Mark Itzkoff
Sent: Monday, January 10, 2011 7:33 AM
To: Mark Itzkoff; 'robert.martin@fda.hhs.gov'
Subject: RE: Pre-Submission Conference for PURAC

Dr. Martin

I am forwarding this request sent last week simply to make certain it was received. If it has already been assigned to a CSO, please let me know who will be co-ordinating the meeting.

Thank you,

Mark Itzkoff

Mark L. Itzkoff
Olsson Frank Weeda Terman Bode Matz PC
Washington, DC 20036
202 518-6327
www.ofwlaw.com

From: Mark Itzkoff
Sent: Tuesday, January 04, 2011 11:19 AM
To: 'robert.martin@fda.hhs.gov'
Subject: Pre-Submission Conference for PURAC

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Dr. Martin

On behalf of our client, PURAC, we are requesting a Pre-submission Conference to review a draft GRAS Notice. The Notice will cover the use of an extended class of ferments, similar to GRN 240, but with a wider range of source material and intended uses.

If possible, we would like to schedule the conference for the week of January 24.

I look forward to speaking with you on this.

Regards,

Mark Itzkoff

Mark L. Itzkoff
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202 518-6327
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Agenda

Pre-Submission Conference

FDA and PURAC, Inc

February 7, 2011

- I. Personal Introductions**
- II. Introduction to PURAC, Inc.**
- III. Description of GRAS Substance, PuraQ™**
 - a. Review of GRASN 240**
 - b. Description of PuraQ™**
 - c. Chemical Composition**
 - d. Manufacturing Process**
 - e. Intended Applications**
- IV. Safety Analysis**
- V. Expert Panel Composition & Conclusion**
- VI. Discussion and Steps Forward**

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000007



November 2, 2012

Richard F. Mann
Keller and Heckman LLP
1001 G Street, NW
Suite 500W
Washington, DC 20001

Re: GRAS Notice No. GRN 000444

Dear Mr. Mann:

The Food and Drug Administration (FDA) has received the notice, dated September 21, 2012, that you submitted on behalf of the American Dairy Products Institute (ADPI) and the U.S. Dairy Export Council (USDEC) 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 September 21, 2012, filed it on September 25, 2012, and designated it as GRN No. 000444.


The subject of the notice is milk protein concentrate and milk protein isolate. The notice informs FDA of the view of ADPI and USDEC that milk protein concentrate and milk protein isolate are GRAS, through scientific procedures, for use as a source of milk protein in meal replacements and bars, term infant formula, milk products, confections and frosting, puddings and fillings, and dressings, soups, sauces, and snack foods.

In accordance with proposed 21 CFR 170.36(f), a copy of the information in this notice that conforms to the information in the GRAS exemption claim (proposed 21 CFR 170.36(c)(1)) is available for public review and copying at www.fda.gov/grasnoticeinventory.

Sincerely yours,

Moraima J. Ramos Valle, M.S.
Division of Biotechnology and
GRAS Notice Review
Center for Food Safety
and Applied Nutrition

(b) (5)



From: [Mann, Richard F.](#)
To: [Ramos-Valle, Moraima](#)
Cc: [Jijon, Alissa D.](#)
Subject: ADPI/USDEC GRAS Notice
Date: Thursday, March 14, 2013 3:47:21 PM

Dear Moraima,

Thanks very much for the very helpful telephone conference yesterday. We have spoken with our clients and they agree that we should withdraw the GRAS notification with the understanding that this will allow us to gather any additional information that you and your colleagues are seeking and re-file the notifications as expeditiously as possible. We will send a formal withdrawal letter to you shortly. In the mea time, we are anxious to proceed with a meeting with the appropriate people at FDA so that we can find out what issues you believe remain to be addressed. Can you please give me some proposed times for a meeting – the sooner the better?

Thanks very much.

Best regards,

Rick Mann and Alissa Jijon
Counsel
American Dairy Products Institute and the U.S. Dairy Export Council

Richard F. Mann
Partner
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From: [Mann, Richard F.](#)
To: [Ramos-Valle, Moraima](#)
Subject: FW: Proposed response to FDA's questions on GRN 444
Date: Tuesday, January 29, 2013 6:17:46 PM

Dear Ms. Ramos Valle:

On behalf of the American Dairy Products Institute (ADPI) and the U.S. Dairy Export Council (USDEC) we hereby respond to your [questions relating to](#) your review of the referenced GRAS Notification for Concentrated Milk Proteins – Milk Protein Concentrate (MPC) and Milk Protein Isolate (MPI). Below we reproduce and respond to each of the questions FDA posed regarding GRAS Notice 444. Where certain questions are inter-related, we indicate as much and provide a consolidated response.

1. Please clearly provide specifications (in addition to typical levels) for the ingredients, including citation to validated methods of analysis for macronutrients (protein, fat, carbohydrate), minerals, and other components.

All specifications discussed in our responses below are determined in accordance with standard laboratory practices and procedures used throughout the dairy industry, including, but not limited to, those described in *Standard Methods for the Examination of Dairy Products* (SMEDP), 17th Ed. (American Public Health Association, 2004).

Typical Composition (%) for Concentrated Milk Proteins

Macronutrient	MPC (%)	MPI (%)
Water	< 6.0	< 6.0
Protein* ^o	≥ 40.0 < 90.0	≥ 90.0
Fat*	< 45.0	< 3.0
Minerals*	< 8.0	< 8.0
Carbohydrate*	< 48.0	< 2.0

* In dry matter

^o N x 6.38

Typical Microbiological Specifications for Concentrated Milk Proteins

Parameter	Specification
Standard Plate Count	< 30,000 cfu/g
Standard Plate Count (products for infant formula only)	< 10,000 cfu/g
<i>Coliform</i>	< 10 cfu/g
<i>E. Coli</i>	< 10 cfu/g
<i>Salmonella</i>	Absent/375 g
Yeast and Molds	< 50 cfu/g
<i>Listeria monocytogenes</i>	Absent/25 g
<i>Staphylococcus aureus</i>	< 10 cfu/g

Typical Product Specifications for Concentrated Milk Proteins

MPC-42	MPC-80
3.5% moisture	3.8% moisture
40.5% protein	77.2% protein
1% milkfat	2.5% milkfat
46% lactose	5.5% lactose
7.9% ash	8.0% ash

- a. Please provide a specification for lead and for *Cronobacter sakazaii*.

Specification for Lead: < 10 ppm

Specification for *Cronobacter Sakazaii*: Neg/375g (infant formula products)

- b. For the specifications for *Salmonella* and *L. monocytogenes*, include the denominator (i.e., in 10 grams or other unit of measure).

Salmonella: Neg/375g

Listeria monocytogenes: Neg/25g

- c. FDA notes that the specification for fat is higher than we might expect even for full fat milk powder. Please compare the specification for total fat with the typical levels (from published data and your proposed ingredient) of fat in full fat milk powder, clarifying where differences (attributed to the methodology or other factor) might occur.

The specification for fat in full fat milk powder is described in 21 C.F.R. § 131.147 ("Dry whole milk") as follows: "It contains not less than 26 percent but less than 40 percent by weight of milkfat on an as is basis." We understand that actual fat content may vary due to minor variations in production methods (see, e.g., 42% milkfat specification described in Codex Alimentarius Commission, ALINORM 99/11, pp. 38, 41, 45 & 49. See also SMEDP at 547, Table 16.15). The maximum specification for fat content provided in GRAS Notice 444 is simply intended to reflect this variability. The fat content specification is not expected to differ significantly from that of full fat milk powder. Please note that MPC also may be manufactured from skim milk, which would result in total fat content well below the specification set forth above.

2. Please provide the source(s) organism of the lactase enzyme, and include a statement that the enzyme is GRAS (or otherwise regulated) for its intended use.

The source organism of the lactase enzyme is *Kluyveromyces lactis*. The lactase enzyme preparation is carried out using current good manufacturing practice, in accordance with 21 C.F.R. § 184.1388 ("Lactase enzyme preparation from *Kluyveromyces lactis*").

3. Please describe the conditions of pasteurization or equivalent processing.

Skim milk or whole milk used in the manufacture of concentrated milk proteins is pasteurized in accordance with the provisions of the Grade "A" Pasteurized Milk Ordinance (PMO) (available at: <http://www.fda.gov/downloads/Food/FoodSafety/Product-SpecificInformation/MilkSafety/NationalConferenceonInterstateMilkShipmentsNCIMSMModelDocuments/UCM291757.pdf>). Alternatively, the concentrated milk proteins are pasteurized after the manufacturing process is complete. Under both approaches, pasteurization is completed in accordance with the time and temperature requirements set forth in Definition FF ("Pasteurization") of the PMO, or, where applicable, any "other process found equivalent to pasteurization for milk and milk products, which has been recognized by FDA as provided in section 403(h)(3) of the FFD&CA." *Id.* at 8.

For ease of reference, we reproduce the PMO's time/temperature guidelines for pasteurization below:

Temperature	Time
63°C (145°F) *	30 minutes
72°C (161°F) *	15 seconds
89°C (191°F)	1.0 second
90°C (194°F)	0.5 seconds
94°C (201°F)	0.1 seconds
96°C (204°F)	0.05 seconds
100°C (212°F)	0.01 seconds

*If the fat content of the milk product is ten percent (%) or greater, or a total solids of 18% or greater, or if it contains added sweeteners, the specified temperature shall be increased by 3°C (5°F).

4. Please provide a statement that the membrane materials used in the manufacturing process are suitable for their intended use, including citation to the relevant regulations for indirect food additives.

The manufacturing techniques employed to concentrate protein and to remove non-protein constituents from milk are based primarily on the use of membrane ultra-filtration technologies. The membranes employed in the production of MPC and MPI comply with the applicable compositional and processing parameters set forth in 21 C.F.R. § 177.2910 ("Ultra-filtration membranes"). Further, the use of such technologies complies with current good manufacturing practices for food-contact applications.

5. Please provide a citation to the method of analysis for melamine and state which components are measured in these analyses for melamine and related compounds.

Analysis is conducted in accordance with ISO/TS 15495:2010 (IDF 230:2010) ("Milk, milk products and infant formulae -- Guidelines for the quantitative determination of melamine and cyanuric acid by LC-MS/MS"), available at http://www.iso.org/iso/catalogue_detail?csnumber=55437. This standard quantitatively evaluates melamine and cyanuric acid content using electrospray ionization liquid chromatography tandem mass spectrometry (LC-MS/MS).

6. FDA notes that the intended use includes infant formula, but there is no information about the safety or general recognition of safety of this ingredient in infant formula (term or preterm); such a discussion might include a comparison of the composition of the ingredient (protein, fat, etc.) with the composition of standard protein and fat sources used in infant formula. Upper use level in formula is high; no basis is given for the levels of use proposed.

MPC and MPI have a long history of safe use in a variety of consumer products, including those marketed for children and infants (both term and pre-term). A representative sample of such products appears below (Source: Innova Database, available at <http://www.foodingredientsfirst.com/innova-database.html>):

Domestic – Infant Products

Manufacturer	Product Description	Ingredient	Launch Date
(b) (4)	(b) (4) Infant Formula	MPI	2012
	Lacto Free Formula, organic	MPC (organic)	2008
	(b) (4) Infant Formula for Fussiness/Gas	MPI	2010

Domestic – Children's Products

Manufacturer	Product Description	Ingredient	Launch Date
(b) (4)	(b) (4) Nutrition Beverage for 1-13	MPC	2012
	Toddler formula	MPC (organic)	2008
	Dairy Snack Dessert for 2 to 4 year olds	MPC	2010
	Flavored yogurt for babies	MPC	2009
	Nutritional beverage for children 1-10	MPC	2003

International – Infant Products

Manufacturer	Product Description	Country	Ingredient	Launch Date
(b) (4)	Infant formula	Germany	MPC	2011
	Pre-term or Low birth weight infant formula	Italy	MPC	2010
	Organic formula for babies (6-9 months)	South Korea	MPC (organic)	2009
	Organic infant formula from birth	Australia	MPC	2006
	(b) (4) formula for 8 months+	Austria	Hydrolyzed MPC	2006

International – Children's Products

Manufacturer	Product Description	Country	Ingredient	Launch Date
(b) (4)	Pediatric Nutrition (b) (4)	Thailand	MPC	2012
	(b) (4) Yogurt for toddlers	Canada	MPC (organic)	2010
	(b) (4) Yogurt	Argentina	MPC	2011
	Pediatric Nutrition (b) (4)	Malaysia	MPC	2010

The sole source ingredients for MPC and MPI (skim or whole milk) are identical to those used to manufacture of milk-derived products appearing in 21 C.F.R. Part 131, and are themselves used in the manufacture of foods as discussed in 21 C.F.R. § 131.110 ("Milk"). As discussed above and in the original submission of GRAS Notice 444 (dated September 21, 2012), the manufacture of MPC and MPI derived from skim or whole milk is conducted in accordance with good manufacturing practices, and in keeping with the standard methods set forth in SMEDP. The well-established safety of the use of milk and milk-based products in children's products and infant formula is thus incorporated by reference into the manufacture of MPC and MPI. The proposed use levels are discussed in further detail in our responses to Questions 8, 9, 10 and 12, below.

7. There is mention that "no pesticides" have been detected, with mention of broad classes of pesticides. There is no discussion of veterinary drug residues or PCBs. As part of the discussion of this issue, include a statement that the milk source material is produced in accordance with good agricultural practices, meets applicable state and federal regulations, and that veterinary drug residues, and levels of PCBs and pesticides are within compliance with all applicable regulations. Further, please provide a discussion where the method of manufacture contributes to removal (or alternatively, concentration) of contaminants. FDA also notes that there are tolerances for various contaminants in milk listed in 21 CFR Part 556 (veterinary drug residues), 21 CFR 109.30 (PCBs), and 40 CFR Part 180 (pesticides). For your information, FDA's action levels for certain contaminants are listed in FDA's Compliance Policy Guide, Section 575.100.

The milk source material is produced in accordance with good agricultural practices, and as such, meets applicable state and federal regulations. Further, veterinary drug residues and levels of PCBs and pesticides comply with all applicable regulations.

More broadly speaking, the raw milk used in the production of MPCs is produced in keeping with the requirements and methodologies set forth in SMEDP, notably Chapter 12 ("Detection of Antibiotic/Drug Residues in Milk and Dairy Products"). Good manufacturing practices include sampling and monitoring programs currently employed by industry to continually test for the contaminants set forth above.

8. Table 5. Typical Levels of Incorporation for MPC and MPI (Section VII.C., p. 13) The level of incorporation for MPC and MPI listed in the table for infant formula is 10-40 g/ 100g as is. Please clarify if the addition levels of 10-40 g of the powder ingredients per 100 g powder form of infant formula is in the finished product.

The level of incorporation is based on the dry mix.

Also, please note the following revised typical levels of incorporation for MPC and MPI in children's foods and beverages, and in infant formula dry mix:

Food Category	Application	Level of Incorporation (grams/100 grams as is)
Nutritional Products	Children's Foods and Beverages*	10-30
Infant Formula Dry Mix	Protein Source in Term and Preterm Infant Formula	10.3-57.9**

* May include follow-on, growing-up (toddler) milk but excludes infant formula

** Depends on protein concentration of MPC/MPI used and assumes MPC/MPI is the only protein source; refers to the level of incorporation in finished infant formula powder (i.e. not reconstituted for consumption)

The responses to questions 9, 10 and 12 are interrelated. Therefore, we reproduce and respond to these questions collectively below.

9. Please explain the notifier's rationale for adding MPC and MPI at the proposed use levels.
10. The notification did not specify whether MPC and MPI are intended for use in term infant formula or both term and preterm infant formulas. Please specify.
12. The amount of protein required in infant formula (21CFR 107.100) is 1.8-4.5 g/100 Cal. According to our calculations*, the proposed maximum use level of MPI (40 g/100 g) will provide an amount of protein greater than 4.5 g/100 Cal, the maximum specified in the regulation, and the proposed minimum use level of MPI (10 g/100 g) will provide an amount of protein slightly less than 1.8 g/100 Cal, the minimum specified in the regulation. Again, according to our calculations*, the proposed minimum use level of MPC (MPC-40) (10 g/100 g) will provide an amount of protein less than 1.8 g/100 Cal, the minimum specified in the regulation. Please explain/clarify.

*The information use for the calculations, came from tables 3 and 4 (nutrient specifications for MPC [MPC-40] and MPI, respectively, p.4), and assuming that MPC (MPC-40) or MPI is the only source of protein in infant formula.

The summary Table 5 that appears in GRAS Notice 444 is intended to provide a general understanding of the potential level of incorporation of MPC or MPI in various food ingredient applications. These values are intended for representative purposes only, and are not intended to serve as specifications for particular products. As discussed in the original submission, the actual level of incorporation will depend on a variety of factors, including, but not limited to, consumer preference, technical feasibility, and existing regulatory requirements. MPC and MPI are intended for use in both term and preterm infant formulas.

For example, as FDA points out, the amount of protein required in infant formula is the subject of 21 C.F.R. § 107.100 ("Nutrient specifications"). Specifically, protein content shall range from 1.8-4.5 g/100 cal. In light of this requirement, we provide the following range of level of incorporation of MPI that would satisfy the protein source requirement set forth above, and confirm that the addition levels are expressed per 100 g powder form of infant formula in the finished product. Assuming, in the case of MPI, a protein content of 90%:

$$(x \text{ g}_{\text{MPI}} / 90\%) \times (1/6.38) \geq 1.8 \text{ g/100 Cal} \rightarrow x \geq 10.34 \text{ g}_{\text{MPI}}$$

$$(x \text{ g}_{\text{MPI}} / 90\%) \times (1/6.38) \leq 4.5 \text{ g/100 Cal} \rightarrow x \leq 25.84 \text{ g}_{\text{MPI}}$$

Therefore, in this specific instance, to meet the requirement set forth in 21 C.F.R. § 107.100, MPI content would range from 10.34-25.84 g/100 Cal. Nevertheless, the ultimate use level for MPC or MPI in products such as infant formula shall be determined on a case-by-case basis by the finished product manufacturer. As with all finished food products for which specific standards of identity exist, ultimately it is the finished product manufacturer's responsibility, not that of the ingredient supplier, to ensure that all applicable limitations are met. This is particularly true where more than one ingredient may contribute a source of nutrients such as fat or protein.

Therefore, in the specific instance detailed above, we would amend the minimum and maximum range for the level of incorporation of MPI accordingly. The specific use level may be adjusted based on the factors discussed above. Ultimately, the finished product manufacturer must ensure that all applicable limitations are met.

Please note that in our response to question 8 above, we amended the range for the level of incorporation of MPC and MPI in infant formula to be 10.3-57.9 grams/100 grams as is.

11. *Lactose Intolerance (Section VII.B.5): The notifier stated that "Because the ratio of carbohydrate and protein in the concentrated milk protein (i.e., 48% / 40% = 1.2 for milk protein concentrate from skim milk, which has the highest level of carbohydrate) is lower than that found in regular milk (i.e., 4.9%/3.% = 1.5 for milk), the use of concentrated milk protein would be expected to result in less lactose intolerance than milk."*

a. *This statement is misleading, lactose intolerance depends on the amount of lactose ingested/consumed (1-2), not ratio of carbohydrate and protein in ingredients. Please clarify.*

b. *Please provide all related references.*

The purpose of this statement is to reflect the understanding that lactose (a carbohydrate) makes up only a fraction of both milk and milk-concentrate products, the balance of which largely consists of protein and fat. As the fraction of protein or fat increases relative to the fraction of carbohydrate, the lactose content is necessarily reduced by weight of the finished product.

Nevertheless, as FDA indicates, lactose intolerance depends on the amount of lactose ultimately ingested or consumed. We do not expect that the lactose tolerability profile of MPC or MPI would differ substantially from that of milk or milk-derived products. Therefore, individuals who exhibit lactose intolerance would approach consumption of products containing MPC or MPI no differently than they would products that contain milk or other milk-derived products (namely, they would limit or restrict such consumption). To that end, finished food products containing MPC or MPI would bear the same allergen labeling information as any product containing milk or milk-derived products to advise the consumer of the presence of lactose-containing ingredients. Notably, many of the products in which MPC or MPI may be incorporated (e.g., spreads, dips, cheese products, yogurt, and dairy beverages) would presumably contain other lactose-containing ingredients as well. Therefore, the finished product manufacturer would employ the same labeling requirements already in place for these products.

See Dairy Research Institute, Lactose Intolerance Scientific Status Report (2011), available at <http://www.nationaldairycouncil.org/Research/ResearchSummaries/Pages/LactoseIntolerance.aspx>.

* * *

We trust that the above responses fully address the Agency's [questions relating](#) to these dairy based ingredients. If you have any further questions, or if we can be of additional assistance, please contact us.

Best regards,

Richard Mann

Counsel to the American Dairy Products Institute and the U.S. Dairy Export Council

Richard F. Mann

Partner

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Writer's Direct Access
Richard F. Mann
(202) 434-4229
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March 18, 2013

Via U.S. Mail and Electronic Mail

Moraima J. Ramos Valle, M.S.
Consumer Safety Officer
Division of Biotechnology and GRAS Notice Review (HFS-225)
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Pkwy
College Park, MD 20740

Re: Request to Withdraw GRN 444 – Amended GRAS Notification
for Concentrated Milk Proteins: Milk Protein Concentrate (MPC)
and Milk Protein Isolate (MPI)

Dear Ms. Ramos Valle:

We respectfully request that the U.S. Food and Drug Administration (FDA) cease to evaluate the Amended GRAS Notification for Concentrated Milk Proteins: Milk Protein Concentrate (MPC) and Milk Protein Isolate (MPI) (GRN 444). We submitted this amended GRAS Notification on September 21, 2012, on behalf of our clients, the American Dairy Products Institute (ADPI) and the U.S. Dairy Export Council (USDEC).

At this time, we would like to withdraw GRN 444 in order to gather additional information regarding the proposed uses of concentrated milk proteins, and we intend to re-file a new GRAS Notification covering these products as soon as practicable.

Thank you for your consideration of this request, and we look forward to receiving confirmation that you have ceased to evaluate GRN 444.

Sincerely,

A handwritten signature in black ink, which appears to be "Richard F. Mann", is written over a large, thick red "X" that is drawn across the signature.

Richard F. Mann
Counsel to the American Dairy Products Institute
and the U.S. Dairy Export Council

Ramos-Valle, Moraima

From: Ramos-Valle, Moraima
Sent: Thursday, April 18, 2013 3:23 PM
To: mann@khlaw.com
Cc: Jijon, Alissa D. (jijon@khlaw.com)
Subject: GRN 444-meeting follow up

Dear Mr. Mann,

This message is to follow up with our meeting yesterday. Please see below a list of comments/questions from our review of GRN 444 and the notifier's responses to our questions received by FDA on January 20, 2013.

As a suggestion, please consider submitting two separate notices for MPC and MPI, one for use in infant formulas and one for use of these ingredients in other foods. Key to this recommendation is that the *intended use of the ingredient* and not the ingredient in and of itself should be the subject of the safety determination.

For infant formula, we note that a resubmission might include the following:

- Focus on general recognition of safety for infant formula; specify whether term or pre-term or both.
 - Discussion of published studies or other publicly-available data that supports the safety of use of the ingredient for the target population (term or pre-term or both).
 - Term and pre-term infants discussed as separate populations, each likely to be consuming different types of formulas.
- Discussions of safety and general recognition of safety of MPC and MPI for their intended uses, based on scientific procedures. A scientific basis summary would include a brief narrative of safety and general recognition of safety of the intended uses of the ingredients, including citation to published references. We note that this basis is distinct from "history of use", which refers to use history of in foods prior to 1958. We note that while a list of products that currently contain the MPC/MPI ingredient (along with a discussion of adverse event reports) may be useful in corroborating published data, such a listing should be accompanied by a comparison of the ingredient that is used in the commercial formula and the ingredient that is the subject of the notice; discussion of composition, manufacture, and use level of the ingredients are relevant points of comparison.
- Clear description of use levels in powdered term or preterm infant formulas, expressed on a per 100 kcal basis.
- Discussion of protein requirements for term or preterm infants (or both).
 - We note protein, fat, and energy needs are not identical for term and preterm infants.^[1] Protein requirements are often expressed on a total ("crude") protein (g total nitrogen x 6.25) or amino-acid based ("true") protein basis; as such, specifications provided for protein should clearly state method used and whether spec is expressed on a total protein or true basis.
 - For infant formula, include discussion of biological value of MPC and MPI—compare with regulatory requirements for infant formula
- Discussion of essential fatty acid and total fat requirements of term or preterm infants or both (see footnote 1)
- For infant formula, include clear discussion of estimated contribution of milk fat to total fat in infant formula and essential fatty acid requirements of infants. Also include discussion of scientific evidence that milk fats support healthy growth of infants. (These are not currently used in infant formulas in the U.S. and there are studies that have reported suboptimal fat absorption for infants fed milk fats—for example, see refs below)

- Williams, ML et al. (1970) Calcium and Fat Absorption in the Neonatal Period. Am J. Clin. Nutr. 23(10): 1322-30.
- Jensen, RG et al. (1978) Lipids of human milk and infant formulas: a review. Am. J. Clin. Nutr. 31: 990-1016.
- Jensen, C et al. (1986) Absorption of individual fatty acids from long chain or medium chain triglycerides in very small infants. Am. J. Clin. Nutr. 43: 745-51.
- Particularly for infant formulas, levels of fat-soluble vitamins (A and D), essential fatty acids (linoleic and linolenic) and residual minerals should be characterized.
- Milk, like other animal sources of fats, may contain fat-soluble contaminants (e.g. dioxins, PCBs, PAHs, pesticides, veterinary drugs). Discussion of good agricultural practices, good manufacturing practices, contaminant specifications and the ability of the method of manufacture to remove (or concentrate) contaminants would be relevant discussion points in the determination of safety of an ingredient containing concentrated milk fat.

For conventional foods, we note that a resubmission might include the following:

- You indicate intended use is as a source of protein in infant formula. What are the intended uses and technical effects in conventional foods?
- General points
 - Fix paragraph at bottom of p. 15 so agent is not making GRAS determination.
 - Tables need clarification whether dry weight basis for all components (see original notice vs. responses)
 - Consider re-evaluation of lead specification, since it is higher than current standards. While the regulation for another milk-based ingredient, whey protein concentrate (WPC; 21 CFR 1979c) includes a specification of 10 ppm heavy metals (as lead) and references FCC version 4, this specification has been updated in subsequent FCCs. Since FCC 5, where the general heavy metals test and limits has been replaced with tests and limits for individual heavy metals, the lead specs have fallen in milk-based ingredients. (Examples: WPC and WPI each NMT 0.5 mg/kg (ppm) lead; casein NMT 1 mg/kg lead).
 - Intended uses in GRAS claim text should match tables of proposed food uses (see Table 5 of GRN 444, missing soups and sauces?) and should not include uses in foods for which standards of identity exist and MPC/MPI are not included as optional ingredients in the standards of identity (e.g. standardized processed cheese).

Components relevant to both infant formula and conventional foods:

- Specifications
 - Specifications should be relevant to articles of commerce. Consider specifications based on protein level. Several published references refer to skim milk powder (35% protein), MPC 42, MPC 50/56, MPC 70/75, MPC 80/85, MPC 90 (or MPI).^[2]
 - We note that one of the notifiers (USDEC), in a fact sheet entitled “What Are Milk Protein Concentrates?”^[3], lists “typical MPCs offered” as MPC₄₂, MPC₇₀, MPC₇₅, MPC₈₀, MPC₈₅, and MPI. In this fact sheet, they show MPC composition for NFDM and MPC products from skim milk (MPC₄₂, MPC₅₆, MPC₇₀, MPC₈₅, MPI).^[4] While there is no regulatory definition for MPC, it appears that the most common industry practice is to use skim milk as a starting material.^[5] If the notice includes both whole milk and skim milk-derived products, distinct sets of specifications from full-fat-milk derived products and skim-milk derived products would be appropriate for representing actual articles in commerce.
 - Methods—indicate whether methods of analyses measure crude protein or “true” protein; total fat or total fatty acids, etc. This is necessary for calculation of nutrient content for infant formula and proportion of fat, specific fatty acids, and protein in formula contributed by MPC.
- Identity of ingredients: Whole, skim, or partially-separated milk
 - You cite standard of identity for dry whole milk (21 CFR 131.147) and cite CODEX reference to milk powders. Perhaps the whole milk-based product would fall under a different name/identity

that reflects ultrafiltered whole milk powder or partially demineralized, reduced lactose whole milk powder?

- Although there is no current statutory definition for milk protein concentrate,^[6] the literature and industry sources predominantly cite skim milk as the starting material for MPC and MPI. The name used for food labeling purposes is under the purview of the CFSAN's Office of Nutrition, Labeling, and Dietary Supplements. However, when published citations, MPC use levels, composition, and other factors are discussed in the notice, the identity of the material (meeting stated specifications) that is the subject of reference should be clearly indicated.
- Are ACR (adjusted casein ratio) products distinct ingredients from "MPC" with respect to identity, use level, intended use, etc.? If yes, notice should clearly discuss.
- Method of manufacture:
 - Please clarify. Are ACR products only from skim milk? Are MPI products only from skim milk?
 - With modifications in method of manufacture and starting materials, what impact is there on the levels of fat-soluble vitamins (A and D), essential fatty acids (linoleic and linolenic), contaminants, and residual minerals?
- Use levels:
 - Separate listings of "as is" products from powdered infant formula or other products that require preparation before consumption.
 - Do use levels and intended technical effects vary with composition of MPC?

Specific comments regarding the responses received on January 29, 2013.

I. Comments/questions on Response (R) to questions (Q) 1-7.

A. R/Q1: In response to Q1 ("Please clearly provide specifications (in addition to typical levels) for the ingredients, including citation to validated methods of analysis for macronutrients (protein, fat, carbohydrate), minerals, and other components."), the notifier provided 3 tables discussed below and stated that "All specifications discussed in our responses" are determined in accordance with standard laboratory practices and procedures used throughout the dairy industry, including, but not limited to, those described in SMEDP, 17th Ed. (American Public Association, 2004). It appears that the notifier did not include/provide citations to all of the methods used. Please provide.

1) The 1st table provided Typical Composition (%) for Concentrated Milk Proteins and the 3rd table provided Typical Product Specifications for "Concentrated Milk Proteins". The notifier provided composition/specifications (specs) for only macronutrients (protein, fat, minerals, water and carbohydrate) in the tables.

•The typical compositions of macronutrients (%) for MPC and MPI (1st table, current amendment) are identical to those for MCP and MPI provided in the Table 1 of the original notification. However, the 1st table (current amendment) indicates that compositions of protein, fat, minerals, and carbohydrate (%) for MPC and MPI are dry matter basis whereas the Table 1 (original notification) indicates that only protein composition is dry matter basis. Clarification is needed. The 3rd table (current amendment) provided macronutrient specs for MPC-42 and MPC-80. Are any of these values dry matter basis?

• FDA noted that the 3rd table included macronutrient specs for MPC-42 and MPC-80 (current amendment) but specs for MPI are not included in the table. Please confirm that the macronutrient specs provided in the Tables 3 and 4 of the original notification are correct specs for MPC-40 and MPI-90, respectively.

•The original notification indicated that the concentrated milk proteins are derived from skim milk, partially separated milk or whole milk from cows. The sources of the ingredients were not stated in the 3rd table (macronutrient specs for MPCs; current amendment) and Tables 3 and 4 (macronutrient specs for MPC-40 & MPI-90; original notification). Please provide 2 tables for the specs: one for MPC and MPI produced from skim milk and one for MPC and MPI produced from whole milk. The specs for MPC-42 and MPC-80 provided in the 3rd table (current amendment) and the specs for MPC-40 and MPI-90 provided in the original notification are confusing. For example, the fat spec for MPC-42 is 1% (3rd table) but

fat spec for MPC-40 (Table 3) is <45%. For another example, the other macronutrient specs (water, protein, minerals & carbohydrate/lactose) for MPC-42 (3rd table) and those for MPC-40 (Table 3) are generally similar even though fat spec for MPC-40 in the Table 3 (original notification) is approx. 45 time greater than that for MPC-42 in the 3rd table (current amendment). All of the macronutrient specs for MPC-42 (3rd table) add up to 99% whereas those for MPC-40 add up to 147%.

- Please provide other nutrient specs for MPI-90 and MPC-40 (in addition to the macronutrient specs).

B. R/Q6: In its response to Q6 (“FDA notes that the intended use includes infant formula, but there is no information about the safety or general recognition of safety of this ingredient in infant formula [term or preterm], such a discussion might include a comparison of the composition of the ingredient [protein, fat, etc.] with the composition of standard protein and fat sources used in infant formula. Upper use level in formula is high; no basis is given for the levels of use proposed.”), the notifier stated that:

1) “MPC and MPI have a long history of safe use in a variety of consumer products, including those marketed for children and infants (both term and pre-term) and provided “A representative sample of such products” (web-site provided), and 2) “The sole source ingredients for MPC and MPI (skim or whole milk) are identical to those used to manufacture of milk-derived products appearing in 21CFR Part 131, and are themselves used in the manufacture of foods as discussed in 21CFR 131.110.” The rotifer also stated that “...the manufacture of MPC and MPI derived from skim or whole milk is conducted in accordance with GMP practices, and in keeping with the standard methods set forth in SMEDP. The well-established safety of the use of milk and milk-based products in children’s products and infant formula is thus incorporated by reference into the manufacture of MPC and MPI.”

FDA has the following comments/questions:

1. History of safe use (see 1 above): the notifier grouped marketed infant formulas and toddler formulas containing MPC/MPI into 4 groups (Domestic-Infant Products, Domestic-Children’s Products, International-Infant Products, and International-Children’s Products); and provided manufacturer’s name, product description, ingredient, and launch date for each product. Although MPI and MPC are intended to be used in both term and preterm infant formula, no US pre-term infant formula group is included. Further, the notifier did not provided any discussion on the information they compiled. The following summarizes the information that the notifier provided:

- Domestic-Infant Products: 3 term infant formulas containing MPC/MPI are marketed in the US. No preterm infant formulas containing MPC/MP are marketed in US. The 3 formulas are (b) (4) (AN), (b) (4) (b) (4) Infant Formula for Fussiness/Gas (AN), and Lacto Free Organic Formula (b) (4) (b) (4). The first 2 formulas (AN) are the same formula. Dr. Tonucci (project manager for (b) (4) (b) (4)) indicated that Lacto Free Organic Formula is toddler formula for toddlers 1 year of age or older, and therefore, it should not be listed under infant formula product. This indicates that 1 term infant formula containing MPC/MPI is marketed in US and that no preterm formula containing the ingredients is marketed in the US.

- International-Infant Products: one “Pre-term or low birth weight infant formula” is marketed in Italy ((b) (4) (b) (4)); launch date, 2010) (total 5 formulas are listed for this category).

- 5 domestic and 5 international children’s products (toddler formula, beverages, dessert, yogurt, etc.) are marketed in US and internationally.

a. According to the notification, the ingredients, MPC and MPI, are manufactured from whole milk, partially separated milk, or skim milk using membrane filtration technologies. Are the ingredients used in the infant products (listed above) manufactured by membrane filtration technologies? What are the sources of MPC and MPI: used in the infant products (skim milk or whole milk)? Please discuss these.

b. The notifier stated that “MPC and MPI have a long history of safe use in a variety of consumer products, including those marketed for children and infants (both term and pre-term). Please discuss the results of the data that the notifier compiled for term and preterm infant formulas containing MPC and MPI (see above).

c. Comparison of the composition of the ingredient (MPC/MPI) with the composition of standard protein and fat sources used in the infant formula are not provided.

II. Comments/Questions on Responses to Questions 8-12

A. R/Q8: In response to the question 8 (“.....Please clarify if the addition levels of 10-40 g of the powder ingredients per 100 g powder form of infant formula is in the finished product.”), the notifier indicated that “The level of incorporation is based on the dry mix.” and provided revised typical levels of incorporation for MPC and MPI in infant formula dry mix (10.3-57.9 g/100 g powder). The footnote to the table indicates that the level of incorporation in finished infant formula powder is 10.3-57.9 g.

B. R/Q9: The notifier did not explain its rationale for adding MPC and MPI at the proposed use level (10-40 g/100 g powder product). Instead they revised/increased the level. See R/Q 8 &12.

C. R/Q10: The notifier indicated that MPC/MPI are intended for use in both term and preterm infant formulas (also see R/Q6). However, the notifier did not provide thorough discussion on the safety of the ingredients for use in term and preterm infant formula. See R/Q6.

D. R/Q12:

1) The amount of protein required in infant formula (21CFR 107.100) is 1.8-4.5 g/100 Cal. According to our calculations*, the proposed maximum use level of MPI (40 g/100 g) will provide an amount of protein greater than 4.5 g/100 Cal, the maximum specified in the regulation, and the proposed minimum use level of MPI (10 g/100 g) will provide an amount of protein slightly less than 1.8 g/100 Cal, the minimum specified in the regulation. Again, according to our calculations*, the proposed minimum use level of MPC (MPC-40) (10 g/100 g) will provide an amount of protein less than 1.8 g/100 Cal, the minimum specified in the regulation. Please explain/clarify.

*The information used for the calculations, came from tables 3 and 4 (nutrient specifications for MPC [MPC-40] and MPI, respectively, p.4), and assuming that MPC (MPC-40) or MPI is the only source of protein in infant formula.

2) The notifier did not explain the rationale for the proposed min and max use levels of MPC/MPI in infant formula. Instead the notifier revised/increased the level of incorporation from 10-40 g/100 g powder to 10.3-57.9 g/100 g powder. The notifier indicated that ultimately it is the finished product manufacturer’s responsibility, not that of the ingredient supplier, to ensure that all applicable limitations are met.

•The notifier provided an equation for calculating the amount of MPI to be added to 100 g powder to meet the protein requirements. Calculation using MPC was not discussed. Please provide calculated amount of MPC (min & max) to be added to the 100 g powder product.

•Our calculation method does not match step for step with that of the notifier’s. However, my calculated values for MPI per 100 g powder product are close to the notifier’s. Please provide explanation for using 6.38 and for multiplying 0.9 (instead of dividing) when calculating MPI addition level per 100 g powder formula. I assume that the level of MPI/MPC addition (10.3-57.9 g) is dry weight. Please confirm.

•Our calculation indicate that 57.9 g of MPI will provide approx. 10 g of protein/100 Cal, much higher than the max amount of protein required (4.5 g/100 Cal). The 57.9 g MPC-40 will provide 4.5 g protein/100 Cal. However, 57.9 g MPC-40 will also provide approx. 5 g fat (milk fat)/100 Cal which is close to the max level of fat required in infant formula (3.3 - 6.0 fat/100 Cal). This does not leave much room for adding other oils/fats in infant formula as the source of linoleic acid (& linolenic acid). Please discuss the notifier’s rationale for adding MPC/MPI at the amended max use level of 57.9 g/100 g powder.

d. R/Q11: Lactose Intolerance

1) Please provide lactose and galactose contents in the MPC after hydrolysis of lactose by lactase enzyme.

2) The 4th line from bottom, last paragraph (p.7): “lactose-containing ingredients” should be milk or milk containing ingredients.

3) Please define/explain “the lactose tolerability profile of MPI or MPC”.

Please note that within our questions/comments are examples, references, and internal calculations, so please feel free to contact me if you have any questions.

It was nice to meet you and your colleagues face to face.

Sincerely,

Moraima

Moraima J. Ramos Valle, M.S.
Consumer Safety Officer
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Office of Food Additive Safety
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U.S. Food and Drug Administration
Phone: (240) 402-1248
Email: Moraima.Ramos-Valle@fda.hhs.gov

^[1] Nutritional requirements for infant formulas are stated in 21 CFR 107.100 and in section 412(i) of the FFDCa. FDA also refers to a 1998 report from the Life Sciences Research Office on the nutrient requirements for infant formulas; this report contains updated recommendations for total fat, protein, and essential fatty acids that are not covered in FDA’s regulations (Final rule 50 FR 45108, October 30, 1985). Information for nutritional requirements of preterm infants (exempt formulas) is discussed elsewhere (see scientific assessment of the LSRO/ASN (American Society for Nutritional Sciences; Klein, C.J. (2002) J. Nutr. 132: 1395-1577.)

^[2] 1) O’Kennedy et al. 2009. Dairy ingredients in non-dairy food systems. *In* Dairy-derived ingredients. Food and nutraceutical uses. (Corredig, M., Editor) CRC Press, Woodhead Publishing, Boca Raton: pp. 482-506; 2) Singh H. Protein interactions and functionality of milk protein products, *IBID* pp. 644-674; 3) Wisconsin Center for Dairy Research and the Wisconsin Milk Marketing Board “Dairy Proteins” http://www.cdr.wisc.edu/programs/dairyingredients/pdf/dairy_proteins.pdf; 4) Wisconsin Center for Dairy Research “Dried Dairy Ingredients” Handbook (see footnote 4); 5) Appendix IV of GAO report (2001) <http://www.gao.gov/products/GAO-01-326> (accessed March 23, 2013); 6) Chandan RC. 2008. Dairy processing and quality assurance: An overview. *In* Dairy Processing and Quality Assurance. (Chandan RC, ed., Kilara A, Shah NP, associate eds.) Wiley-Blackwell, Iowa: pp. 1-40; 7) Rollemma HS and Muir DD. 2009. Casein and related products. *In* Dairy Powders and Concentrated Products (Tamime AY, ed.), Wiley-Blackwell, UK: pp. 235-254; 8) Deeth HC and Hartano J. Chemistry of milk-Role of constituents in evaporation and drying. *Ibid.* pp. 1-27.

^[3] www.innovatewithdairy.com “Milk Protein Concentrate Sell Sheet” under Featured Articles/Research http://www.innovatewithdairy.com/SiteCollectionDocuments/Milk%20Protein%20Concentrate%20Sell%20Sheet_%20FINAL.pdf (Accessed March 26, 2013)

^[4] Source: Smith, K. Dried Dairy Ingredients. Wisconsin Center for Dairy Research. Available at: http://www.cdr.wisc.edu/programs/dairyingredients/pdf/dried_dairy_ingdients.pdf. Accessed May 18, 2002 by USDEC (March 23, 2013 by FDA).

^[5] Cessna, J. Milk Protein Products and Related Government Policy Issues. USDA/AMS, Feb 2004. <http://www.ams.usda.gov/AMSV1.0/getfile?dDocName=STELDEV3004526>; GAO Report. Imports, Domestic Production, and Regulation of Ultra-Filtered Milk. March 2001. GAO-01-326. <http://www.gao.gov/products/GAO-01-326>

^[6] Cessna, J. Milk Protein Products and Related Government Policy Issues. USDA/AMS, Feb 2004. <http://www.ams.usda.gov/AMSV1.0/getfile?dDocName=STELDEV3004526>; GAO Report. Imports, Domestic Production, and Regulation of Ultra-Filtered Milk. March 2001. GAO-01-326. <http://www.gao.gov/products/GAO-01-326>

From: [Mann, Richard F.](#)
To: [Ramos-Valle, Moraima](#)
Subject: RE: GRN 444
Date: Monday, January 14, 2013 8:38:49 AM

Dear Ms. Ramos Valle,

I apologize for the delay. We anticipate sending you responses to your questions this week.

Best regards,

Rick Mann

Counsel to the American Dairy Products Institute and the U.S. Dairy Export Council

Richard F. Mann
Partner

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From: Ramos-Valle, Moraima [mailto:Moraima.Ramos-Valle@fda.hhs.gov]
Sent: Wednesday, January 09, 2013 11:27 AM
To: Mann, Richard F.
Subject: RE: GRN 444

Dear Mr. Mann,

I just wanted to follow up with your email below. Could you please provide me a timeframe on when will you be able to submit the responses? This will help me with the time management for this review.

Thanks,
Moraima

Moraima J. Ramos Valle, M.S.

Consumer Safety Officer

Division of Biotechnology and GRAS Notice Review

Food and Drug Administration

Phone: 240-402-1248

Email: Moraima.Ramos-Valle@fda.hhs.gov

From: Mann, Richard F. [<mailto:Mann@khlaw.com>]
Sent: Monday, December 17, 2012 5:29 PM
To: Ramos-Valle, Moraima
Subject: RE: GRN 444

Dear Ms. Ramos,

We are gathering the requested information and should have a full response back to you shortly.

In the meantime, please contact me with any additional questions.

Best regards,

Rick Mann
Counsel to the American Dairy Products Institute and the U.S. Dairy Export Council

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From: Ramos-Valle, Moraima [<mailto:Moraima.Ramos-Valle@fda.hhs.gov>]
Sent: Wednesday, November 21, 2012 1:48 PM
To: Mann, Richard F.
Cc: Ramos-Valle, Moraima
Subject: GRN 444

Dear Mr. Mann,

As we move forward with our review of GRAS Notice 444, we have identified the following questions/clarifications:

1. Please clearly provide specifications (in addition to typical levels) for the ingredients, including citation to validated methods of analysis for macronutrients (protein, fat, carbohydrate), minerals, and other components.
 - a. Please provide a specification for lead and for *Chronobacter sakazakii*.
 - b. For the specifications for *Salmonella* and *L.monocytogenes* , include the denominator (i.e. in 10 grams or other unit of measure).
 - c. FDA notes that the specification for fat is higher than we might expect even for full fat milk powder. Please compare the specification for total fat with the typical levels (from published data and your proposed ingredient) of fat in full fat milk powder, clarifying where differences (attributed to the methodology or other factor) might occur.
2. Please provide the source(s) organism of the lactase enzyme, and include a statement that the enzyme is GRAS (or otherwise regulated) for its intended use.
3. Please describe the conditions of pasteurization or equivalent processing.
4. Please provide a statement that the membrane materials used in the manufacturing process are suitable for their intended use, including citation to the relevant regulations for indirect food additives.
5. Please provide a citation to the method of analysis for melamine and state which components are measured in these analyses for melamine and related compounds.
6. FDA notes that the intended use includes infant formula, but there is no information about the safety or general recognition of safety of this ingredient in infant formula (term or preterm); such a discussion might include a comparison of the composition of the ingredient (protein, fat, etc) with the composition of standard protein and fat sources used in infant formula. Upper use level in formula is high; no basis is given for the levels of use proposed.
7. There is mention that "no pesticides" have been detected, with mention of broad classes of pesticides. There is no discussion of veterinary drug residues or PCBs. As part of the discussion of this issue, include a statement that the milk source material is produced in accordance with good agricultural practices, meets applicable state and federal regulations, and that veterinary drug residues, and levels of PCBs and pesticides are within compliance with all applicable regulations. Further, please provide a discussion where the method of manufacture contributes to removal (or alternatively, concentration) of contaminants. FDA also notes that there are tolerances for various contaminants in milk listed in 21 CFR Part 556 (veterinary drug residues), 21 CFR 109.30 (PCBs), and 40 CFR Part 180 (pesticides). For your information, FDA's action levels for certain contaminants are listed in FDA's Compliance Policy Guide Section 575.100.
8. Table 5. Typical Levels of Incorporations for MPC and MPI (Section VII.C, p.13)
The level of incorporation for MPC and MPI listed in the table for infant formula is 10-40 g/100 g as is. Please clarify if the addition levels of 10-40 g of the powder ingredients per 100 g powder form of infant formula is in the finished product.
9. Please explain, the notifier's rationale for adding MPC and MPI at the proposed use levels.

10. Intended Use: The notification did not specify whether MPC and MPI are intended for use in term infant formula or both term and preterm infant formulas. Please specify.
11. Lactose Intolerance (Section VII.B.5): The notifier stated that “Because the ratio of carbohydrate and protein in the concentrated milk protein (i.e., 48% / 40% =1.2 for milk protein concentrate from skim milk, which has the highest level of carbohydrate) is lower than that found in regular milk (i.e., 4.9%/3.%=1.5 for milk¹⁶), the use of concentrated milk protein would be expected to result in less lactose intolerance than milk.”
- a. This statement is misleading, lactose intolerance depends on the amount of lactose ingested/consumed (1-2), not ratio of carbohydrate and protein in ingredients. Please clarify.
 - b. Please provide all related references.
12. The amount of protein required in infant formula (21CFR 107.100) is 1.8-4.5 g/100 Cal. According to our calculations*, the proposed maximum use level of MPI (40 g/100 g) will provide an amount of protein greater than 4.5 g/100 Cal, the maximum specified in the regulation, and the proposed minimum use level of MPI (10 g/100 g) will provide an amount of protein slightly less than 1.8 g/100 Cal, the minimum specified in the regulation. Again, according to our calculations*, the proposed minimum use level of MPC (MPC-40) (10 g/100 g) will provide an amount of protein less than 1.8 g/100 Cal, the minimum specified in the regulation. Please explain/clarify.
- *The information use for the calculations, came from tables 3 and 4 (nutrient specifications for MPC [MPC-40] and MPI, respectively, p.4), and assuming that MPC (MPC-40) or MPI is the only source of protein in infant formula.

Please feel free to contact me if you have any questions.

Sincerely,

Moraima J. Ramos Valle, M.S.

Consumer Safety Officer

Division of Biotechnology and GRAS Notice Review

Food and Drug Administration

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